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| <p>(54) Title: PAPILLOMAVIRUS POLYPROTEIN CONSTRUCTS</p> | | |
| <p>(57) Abstract</p> <p>A papillomavirus polyprotein construct comprises at least two amino acid sequences fused directly or indirectly together, each of the sequences being the sequence of an early ORF protein of papillomavirus or an immunogenic variant or fragment thereof, and at least one of said sequences being other than the E6 or E7 protein sequence or an immunogenic variant or fragment thereof. Nucleic acid molecules encoding the polyprotein construct, prophylactic or therapeutic compositions comprising the polyprotein construct or the nucleic acid molecule, and methods for eliciting an immune response against papillomavirus in a host animal are also provided.</p> | | |
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"PAPILLOMAVIRUS POLYPROTEIN CONSTRUCTS"

FIELD OF THE INVENTION

5 This invention relates to polyprotein constructs and in particular polyprotein constructs comprising a plurality of papillomavirus (PV) amino acid sequences which may be used in compositions for eliciting an immune response against PV, and particularly human papillomavirus (HPV), in a host animal.

10 BACKGROUND OF THE INVENTION

Papillomaviruses induce benign hyperproliferative lesions in humans and in many animal species, some of which undergo malignant conversion. The biology of papillomavirus infection is summarised in a review by J.P. Sundberg, entitled
15 "Papillomavirus Infections in Animals" In "Papillomaviruses and Human Disease" edited by K. Syrjanen, L. Gissmann and L.G. Koss, Springer Verlag (1987).

Papillomaviruses are a family of small DNA viruses encoding up to eight early (E1, E2, E3, E4, E5, E6, E7 and E8) and two late genes (L1 and L2). These viruses have been
20 classified in several distinct groups such as HPV which are differentiated into types 1 to ~70 depending upon DNA sequence homology. A clinicopathological grouping of HPV and the malignant potential of the lesions with which they are most frequently associated are summarised in "Papillomaviruses and Human Cancer" by H. Pfister, CRC Press, Inc. (1990). For example, HPV type 1 (HPV-1) is present in plantar warts, HPV-6 or HPV-11
25 are associated with condylomata acuminata (anogenital warts), and HPV-16 or HPV-18 are common in pre-malignant and malignant lesions of the cervical squamous epithelium.

The immunological approach to the prevention of HPV disease requires a thorough analysis of the viral proteins against which humoral and cellular immune
30 responses are mounted during and after infection. However, despite recent limited

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success (Kreider *et al.*, 1986, *J. Virol.*, **59**, 369; Sterling *et al.*, 1990, *J. Virol.*, **64**, 6305; Meyers *et al.*, 1992, *Science*, **257**, 971; Dollard *et al.*, 1992, *Genes and Development*, **6**, 1131), papillomaviruses are notoriously refractory to growth in cultured cells (Teichman and LaPorta, 1987 *In "The Papovaviridae"*, Vol 2 edited by N.P. Salzman and P.M. Howley, p.109). As a consequence, the lack of viral reagents has delayed the analysis of the immune response to PV infection.

The recent advent of recombinant expression systems *in vitro* has allowed the production of viral proteins encoded by both early and late genes in relatively large amounts and in a purified form (Tindle *et al.*, 1990, *J. Gen. Virol.*, **71**, 1347; Jarrett *et al.*, 1991, *Virology*, **184**, 33; Ghim *et al.*, 1992, *Virology*, **190**, 548; Stacey *et al.*, 1991, *J. Gen. Virol.*, **73**, 2337). These systems have, for the first time, allowed the analysis of the host immune response to these viral proteins.

Interest in immune responses to the non-structural early open reading frame (ORF) proteins of HPV has centred on HPV-16 E7 because of an apparent association between serum antibodies to this protein and cervical cancer (for a review, see "Immune Response to Human Papillomaviruses and the Prospects of Human Papillomavirus-Specific Immunisation" by Tindle and Frazer *In "Human Pathogenic Papillomaviruses"* edited by H. zur Hausen, Current Topics in Microbiology Immunology, **186**, Springer-Verlag, Berlin, 1994).

The immune responses to other HPV early ORF proteins have also been investigated including HPV-16 E6 (Stacey *et al.*, 1992, *J. Gen. Virol.*, **73**, 2337; Bleul *et al.*, 1991, *J. Clin. Microbiol.*, **29**, 1579; Dillner, 1990, *Int. J. Cancer*, **46**, 703; and Müller *et al.*, 1992, *Virology*, **187**, 508), HPV-16 E2 (Dillner *et al.*, 1989 *Proc.Natl. Acad. Sci.USA*, **86**, 3838; Dillner, 1990, *supra*; Lehtinen *et al.*, 1992, *J. Med. Virol.*, **37**, 180; Mann *et al.*, 1990, *Cancer Res.*, **50**, 7815; and Jenison *et al.*, 1990, *J. Infect. Dis.*, **162**, 60) and HPV-16 E4 (Köchel *et al.*, 1991, *Int. J. Cancer*, **48**, 682; Jochmus-Kudielka *et al.*, 1989, *JNCI*, **81**, 1698; and Barber *et al.*, 1992, *Cancer Immunol. Immunother.*, **35**,

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33). However, comparison of these studies reveals a lack of correlation between the results of the various assays which have been used in assessing HPV early ORF protein reactivity in serum (Tindle and Frazer, 1994, *supra*).

5 In addition, antibodies to other HPV early ORF proteins have not yet been sought with sufficient rigour in large enough numbers of patients to determine their utility as disease markers or as indicators of HPV protein immunogenicity following HPV infection.

A problem associated with immunising animals with preparations of individual PV
10 proteins is that most of these proteins are comparatively small and might therefore not comprise many reactive epitopes. In addition, immunodominance of particular B or T cell epitopes within a single PV protein would vary presumably between animals of different major histocompatibility (MHC) backgrounds. To this end, the efficacy of such immunogens, in respect of eliciting an immune response against PV, might be expected
15 to differ between animals of diverse MHC background.

In addition, there is surprisingly little knowledge regarding which PV proteins are expressed by infected cells at various stages of differentiation, and hence it is not possible to predict which proteins will be responsible for defining appropriate immunological
20 targets.

The present invention provides a polyprotein construct comprising a plurality of PV early ORF proteins in one fused or linked construct to improve the efficacy of immune stimulation against PV infection and to avoid the need to define specific immunological
25 targets.

SUMMARY OF THE INVENTION

In one aspect, the present invention provides as an isolated product, a polyprotein
30 construct comprising at least two amino acid sequences fused directly or indirectly

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together, each of said sequences being the sequence of an early open reading frame (ORF) protein of papillomavirus (PV) or an immunogenic variant or fragment thereof, and at least one of said sequences being other than the E6 or E7 protein sequence or an immunogenic variant or fragment thereof.

5

In yet another aspect, the present invention provides a composition for eliciting a humoral and/or cellular immune response against PV in a host animal, said composition comprising an immunologically effective amount of a construct as described above, together with a pharmaceutically acceptable carrier and/or diluent.

10

In yet another aspect, this invention provides a method for eliciting a humoral and/or cellular response against PV in a host animal, which method comprises administering to the host animal an immunologically effective amount of a polyprotein construct as described above. In a related aspect, the invention also extends to use of
15 such a polyprotein construct in eliciting an immune response against PV in a host animal. Preferably, the host animal is a human, however the host animal may also be a non-human mammal.

The present invention also extends to a nucleic acid molecule which encodes a
20 polypeptide construct as broadly described above. Such a nucleic acid molecule may be delivered to a host animal in a nucleic acid vaccine composition with a pharmaceutically acceptable carrier and/or diluent, for expression of the encoded polyprotein construct *in vivo* in a host animal. Alternatively, the nucleic acid molecule may be included in a recombinant DNA molecule comprising an expression control sequence operatively
25 linked to the nucleic acid molecule.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of
30 integers but not the exclusion of any other integer or group of integers."

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DETAILED DESCRIPTION OF THE INVENTION

The term "polyprotein construct" as used herein is used to describe a protein construct made up of individual proteins that have been joined together in a sequence
5 whereby they retain their original relevant biological activities.

The term "isolated" as used herein denotes that the polyprotein construct has undergone at least one purification or isolation step, and preferably is in a form suitable for administration to a host animal.

10

By use of the term "immunologically effective amount" herein in the context of treatment of PV infection, it is meant that the administration of that amount to an individual PV infected host, either in a single dose or as part of a series, that is effective for treatment of PV infection. By the use of the term "immunologically effective amount"
15 herein in the context of prevention of PV infection, it is meant that the administration of that amount to an individual host, either in a single dose or as part of a series, that is effective to delay, inhibit, treat or prevent PV infection or disease. The effective amount varies depending upon the health and physical condition of the individual to be treated, the taxonomic group of individual to be treated, the capacity of the individual's immune
20 system to synthesise antibodies, the degree of protection desired, the formulation of the immunogen, the assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials.

25 Preferably, the amino acid sequences in the polyprotein construct substantially correspond to the sequences of wild-type early ORF proteins of PV, including allelic or other variants thereof. Suitable variants include variants having single or multiple amino acid substitutions or additions to the wild-type sequences, and may have at least 50-60%, more preferably at least 70-80%, and most preferably at least 90%, similarity to the wild-
30 type amino acid sequences, provided the variant is capable of eliciting an immune

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response against PV in a host animal. The amino acid sequences may also be immunogenic fragments of the wild-type early ORF proteins, that is fragments of the proteins which are capable of eliciting an immune response in a host animal. Suitably, the immunogenic fragment will comprise at least five, and more preferably at least ten, 5 contiguous amino acid residues of the particular protein. Such immunogenic fragments may also be recognised by PV-specific antibodies, particularly antibodies which have a protective or therapeutic effect in relation to PV infection. Preferably, the immunogenic fragment is a non-full length fragment of a wild-type amino acid sequence, which may for example comprise a deletion mutant of an early ORF protein corresponding to at least 10 50%, more preferably 60-70%, and even 80-90% of the full length wild-type amino acid sequence.

The amino acid sequences in the polyprotein construct of the present invention may be selected from the group consisting of the E1, E2, E3, E4, E5 (E5a, E5b), E6, E7 and 15 E8 proteins of PV, and may be included in the construct in any desired order. By way of example, the construct may be selected from the group consisting of:

- (a) E6/E4
- (b) E6/E5a/E4
- (c) E6/E7/E4
- 20 (d) E6/E7/E5a/E4
- (e) E6/E7/E1/E4
- (f) E6/E7/E5a/E1/E4
- (g) E6/E7/E5a/E1/E2/E4
- (h) E6/E7/E5a/E5b/E1/E2/E4
- 25 (i) E2/E5b
- (j) E2/E1/E5b
- (k) E2/E5a/E5b
- (l) E2/E1/E5a/E5b
- (m) E2/E4/E5a/E5b/E6/E7/E1
- 30 (n) E2/E3/E4/E5/E8/E6/E7/E1.

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As described above, at least one of the early ORF proteins is other than the E6 or E7 proteins. Preferably one of the early ORF proteins in the construct is the E4 protein.

The polyprotein constructs of this invention preferably comprise at least three, and
5 more preferably three, four or five early ORF protein sequences. In addition, two or more different polyprotein constructs based on different combinations of early ORF proteins and/or different PV genotypes may be included in a single composition for prophylactic or therapeutic use.

10 In the polyprotein constructs of this invention, the amino acid sequences may be fused or linked directly together. Alternatively, they may be linked with a linker sequence of from 1 to 50, preferably 1 to 20, and more preferably 1 to 5, amino acid residues between the separate amino acid sequences. By way of example, such a linker sequence may be an amino acid sequence encoded by the nucleotide sequence
15 comprising a restriction endonuclease site. Linker sequences as described above may also be provided before and/or after the amino acid sequences in the polyprotein constructs.

The polyprotein constructs of this invention may also comprise a tag protein or
20 peptide moiety fused or otherwise coupled thereto to assist in purification of the polyprotein construct. Suitable tag moieties include, for example, (His)₆, glutathione-S-transferase (GST) and FLAG (International Biotechnologies), with the (His)₆ tag moiety being preferred. The constructs may further comprise a component to enhance the immunogenicity of the polyprotein. The component may be an adjuvant such as
25 diphtheria or cholera toxin or *E. coli* heat labile toxin (LT), or a non-toxic derivative thereof such as the holotoxoid or B subunit of cholera toxin or LT. In addition, the polyprotein construct of the invention may comprise a lipid binding region to facilitate incorporation into ISCOMs. Suitable lipid binding regions are disclosed by way of example in Australian Provisional Patent Application No. PN8867/96, dated 25 March
30 1996. A preferred lipid binding region is an influenza haemagglutinin tail.

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The present invention also provides a nucleic acid molecule comprising a sequence of nucleotides which encodes a polyprotein construct as broadly described above.

5 The nucleic acid molecule may be RNA or DNA, single stranded or double stranded, in linear or covalently closed circular form. It will be appreciated that the sequence of nucleotides of this aspect of the invention may be obtained from natural, synthetic or semi-synthetic sources; furthermore, this nucleotide sequence may be a naturally-occurring sequence, or it may be related by mutation, including single or
10 multiple base substitutions, deletions, insertions and inversions, to such a naturally-occurring sequence, provided always that the nucleic acid molecule comprising such a sequence is capable of being expressed as a polyprotein construct as described herein.

The nucleotide sequence may have expression control sequences positioned
15 adjacent to it, such control sequences being derived from either a homologous or a heterologous source.

Since nucleic acid molecules may be delivered directly as "naked DNA" to a host animal, (see, for example, Wolfe *et al.*, 1990, *Science* 247:1465 and Fynan *et al.*, 1993,
20 *Proc.Natl. Acad. Sci. USA*, 90:11478), the present invention also includes a nucleic acid vaccine composition comprising a nucleic acid molecule as described above, together with a pharmaceutically acceptable carrier and/or diluent.

Immunisation with an isolated nucleic acid molecule allows *in vivo* synthesis of
25 the encoded polyprotein construct by the host animal in a manner similar to the manner in which PV proteins are expressed during infection by PV. In this aspect, the present invention also extends to a method for eliciting an immune response against PV in a host animal, which method comprises administering to the host animal an immunologically effective amount of a nucleic acid molecule as described above. The invention also

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extends to use of such a nucleic acid molecule in eliciting an immune response against PV in a host animal.

This invention also provides a recombinant DNA molecule comprising an
5 expression control sequence having promoter and initiator sequences, the nucleotide
sequence encoding the polyprotein construct being located 3' to the promoter and
initiator sequences and a terminator sequence located 3' to this sequence of nucleotides.
In yet another aspect, the invention provides a recombinant DNA cloning vehicle such
as a plasmid capable of expressing the polyprotein construct, as well as a host cell
10 containing a recombinant DNA cloning vehicle and/or a recombinant DNA molecule as
described above.

Suitable expression control sequences and host cell/cloning vehicle combinations
are well known in the art, and are described by way of example, in Sambrook *et al.*
15 (1989) Molecular Cloning : A Laboratory Manual, 2nd ed. Cold Spring Harbor, New
York, Cold Spring Harbor Laboratory Press. Thus, the nucleotide sequence may be
ligated into any suitable expression vector, which may be either a prokaryotic or
eukaryotic expression vector. Preferably, the vector is a prokaryotic expression vector
such as pTrcHisA or pGEX-STOP (a pGEX expression vector (Amrad/Pharmacia Biotech)
20 which has been manipulated so as to result in truncation of the GST moiety, disclosed in
Australian Provisional Patent Application No. PN8272/86, dated 26 February 1996).
Whilst the host cell is preferably a prokaryotic cell, more preferably a bacterium such as
E. coli, it will be understood that the host cell may alternatively be a yeast or other
eukaryotic cell, or insect cells infected with baculovirus or the like.

25

Once recombinant DNA cloning vehicles and/or host cells expressing a
polyprotein construct of this invention have been identified, the expressed polypeptides
synthesised by the host cells, for example, as a fusion protein, can be isolated
substantially free of contaminating host cell components by techniques well known to
30 those skilled in the art.

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The polyprotein construct-encoding DNA sequence is formed by linking or "fusing" sequences encoding each of the individual protein moieties. The first sequence in the polyprotein DNA construction has a promoter element and a ribosome binding site. These elements assure that transcription of the polyprotein DNA into mRNA begins at a defined site and that the signal, the ribosome binding site, needed for translation of mRNA into protein is present. Synthesis of the polyprotein is made continuous from one protein component to the next by removing or altering any initiation or binding signals and stop codons from the subsequent protein-encoding sequences. The stop codon, normally a signal for the ribosome to stop translation and to end the polypeptide, is not altered or removed from the last DNA sequence. The individual protein encoding sequences are jointed such that a proper phasing is made of the mRNA reading frames for translation of the sequence into the desired amino acids. Once a DNA sequence encoding a polyprotein construct or a "polyprotein gene" is made, it is necessary to demonstrate that the construction leads to production of a stable polyprotein construct. If the resulting protein is not stable, for example because the junctions between the proteins are vulnerable to proteolytic digestion, then the junction regions are modified. This can be done by inserting different amino acids at or near the junction or by building spacers of amino acids between the individual proteins. Linkers or spacers can also be introduced to modify the overall activity of the polyprotein. By adjusting the space between and orientation of the individual proteins it is possible to modify the total activity of the polyprotein construct. Further details of the preparation of polyprotein constructs of the present invention by recombinant DNA techniques are disclosed, by way of example, in US Patent No. 4774180, the disclosure of which is incorporated herein by reference.

25

Preferably, the polymerase chain reaction (PCR) is used to amplify the nucleotide sequences encoding each of the individual PV early ORF proteins. The nucleotide sequences which are amplified may be full length or non full-length fragments thereof. Restriction endonuclease sites may be incorporated in the oligonucleotide primers used for PCR to furnish directional ligation of the amplification products in the same

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translational frame and to enable directional cloning into a suitable expression vector. The primers may encode an artificial initiator codon or a termination codon.

The first nucleotide sequence has an initiator codon. This initiator codon may
5 either be the normal wild-type initiator codon of the first sequence or may be inserted artificially at another chosen position of this sequence. Synthesis of the polyprotein construct is made continuous from one protein component to the next by removing or altering any initiation or binding signals and termination codons. The termination codon must be present in the last nucleotide sequence. This is effected normally by not altering
10 or removing the termination codon of the last nucleotide sequence. However, this termination codon may be inserted artificially, by methods known to persons skilled in the art, by first removing the normal, wild-type termination codon of the last nucleotide sequence and inserting another, in the correct reading frame, at another position of this sequence.

15

The polyprotein construct-encoding DNA sequence may incorporate restriction sites at the flanking ends to facilitate insertion of the DNA sequence into a suitable expression vector.

20 The PV can be a human or an animal PV, and is preferably HPV. The HPV may be of any genotype, and may for example be selected from the group consisting of HPV-6, HPV-11, HPV-16, HPV-18, HPV-33, HPV-35, HPV-31 and HPV-45. Preferably, the HPV is HPV-6 or HPV-11.

25 The present invention is particularly, but not exclusively, directed to polyprotein constructs comprising early ORF proteins of the HPV-6 and HPV-11 genotypes which are causative agents of condylomata acuminata, however it will be appreciated that the invention extends to variants of the corresponding proteins in other HPV genotypes, particularly the HPV-16 and HPV-18 genotypes, and other genotypes which have
30 oncogenic potential of a type similar to HPV-16 and HPV-18.

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The polyprotein constructs of the present invention may comprise early ORF proteins of a single HPV genotype, or alternatively they may comprise early ORF proteins from more than one HPV genotype. In addition, a combination of more than one polyprotein construct may be used in cases where not all early ORF proteins are
5 represented in the one polyprotein construct, or where immune responses to more than one HPV genotype are desired.

The polyprotein constructs of the present invention are provided as isolated proteins, that is they are substantially free of other PV proteins, and find particular utility
10 for the treatment of genital warts, cervical cancer or other conditions caused by HPV in man. The polyprotein constructs can be included in pharmaceutical compositions for the treatment or prevention of diseases involving HPV as well as the other conditions discussed above.

15 The polyprotein constructs of the invention may be used to raise antibodies and/or induce cellular immune responses, either in subjects for which protection against infection by PV is desired, i.e. as prophylactic vaccines, or to heighten the immune response to an PV infection already present, i.e. as therapeutic vaccines. They also can be injected into production species to obtain antisera. In lieu of the polyclonal antisera
20 obtained in the production species, monoclonal antibodies may be produced using the standard methods or by more recent modifications thereof by immortalising spleen or other antibody-producing cells for injection into animals to obtain antibody-producing clones. The polyclonal or monoclonal antibodies obtained, corrected if necessary for species variations, can also be used as therapeutic agents.

25

Direct administration of the polyprotein constructs to a host animal such as a human can confer either protective immunity against PV or, if the subject is already infected, a boost to the subject's own immune response to more effectively combat the progress of the PV induced disease.

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The magnitude of the prophylactic or therapeutic dose of a polyprotein constructs of this invention will, of course, vary with the group of patients (age, sex, etc.), the nature or the severity of the condition to be treated and with the particular polyprotein construct and its route of administration. In general, the weekly dose range for use lies within the
5 range of from about 0.1 to about 5 μg per kg body weight of a mammal.

Any suitable route of administration may be employed for providing a mammal, especially a human, with an effective dosage of a polyprotein construct of this invention. For example, oral, rectal, vaginal, topical, parenteral, ocular, nasal, sublingual, buccal,
10 intravenous and the like may be employed. Dosage forms include tablets, troches, dispersions, suspensions, solutions, capsules, creams, ointments, suppositories, aerosols and the like. Said dosage forms also include injected or implanted slow releasing devices specifically designed for this purpose or other forms of implants modified to additionally act in this fashion.

15

If the polyprotein constructs are to be administered as vaccines, they are formulated according to conventional methods for such administration to the subject to be protected. The polyprotein constructs may be delivered in accordance with this invention in ISCOMSTM (immune stimulating complexes), liposomes or encapsulated in
20 compounds such as acrylates or poly(DL-lactide-co-glycoside) to form microspheres. They may also be incorporated into oily emulsions and delivered orally.

Other adjuvants, as well as conventional pharmaceutically acceptable carriers, excipients, buffers or diluents, may also be included in vaccine compositions of this
25 invention. Generally, a vaccine composition in accordance with the present invention will comprise an immunologically effective amount of the polyprotein construct, and optionally an adjuvant, in conjunction with one or more conventional pharmaceutically acceptable carriers and/or diluents. An extensive though not exhaustive list of adjuvants can be found in Coulter and Cox, "Advances in Adjuvant Technology and Application",
30 in *Animal Parasite Control Utilizing Biotechnology*, Chapter 4, Ed. Young, W.K., CRC

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Press, 1992. As used herein "pharmaceutically acceptable carriers and/or diluents" include any and all solvents, dispersion media, aqueous solutions, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art and
5 is described by way of example in *Remington's Pharmaceutical Sciences*, 18th Edition, Mack Publishing Company, Pennsylvania, U.S.A.

In practical use, a polyprotein construct of this invention can be combined as the active ingredient in intimate admixture with a pharmaceutical carrier according to
10 conventional pharmaceutical compounding techniques. The carrier may take a wide variety of forms depending on the form of preparation desired for administration, e.g. oral or parenteral (including intravenous and intra-arterial). In preparing the compositions for oral dosage form, any of the usual pharmaceutical media may be employed, such as, for example, water glycols, oils, alcohols, flavouring agents, preservatives, colouring agents
15 and the like in the case of oral liquid preparations, such as, for example, suspensions, elixirs and solutions; or carriers such as starches, sugars, microcrystalline cellulose, diluents, granulating agents, lubricants, binders, disintegrating agents and the like in the case of oral solid preparations such as, for example, powders, capsules and tablets. Because of their ease of administration, tablets and capsules represent the most
20 advantageous oral dosage unit form, in which case solid pharmaceutical carriers are obviously employed. If desired, tablets may be sugar-coated or enteric-coated by standard techniques.

In addition to the common dosage forms set out above, the polyprotein constructs
25 of this invention may also be administered by controlled release means and/or delivery devices, including by way of example, the controlled release preparations disclosed in International Patent Specification No. PCT/AU93/00677 (Publication No. WO 94/15636).

Pharmaceutical compositions of the present invention suitable for oral or
30 parenteral administration may be presented as discrete units such as capsules, cachets or

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tablets each containing a predetermined amount of the active ingredient, as a powder or granules or as a solution or a suspension in an aqueous liquid, a non-aqueous liquid, an oil-in-water emulsion or a water-in-oil liquid emulsion. Such compositions may be prepared by any of the methods of pharmacy but all methods include the step of bringing
5 into association the active ingredient with the carrier which constitutes one or more necessary ingredients. In general, the compositions are prepared by uniformly and intimately admixing the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product into the desired presentation.

10 Further features of the present invention are more fully described in the following Example(s). It is to be understood, however, that this detailed description is included solely for the purposes of exemplifying the present invention, and should not be understood in any way as a restriction on the broad description of the invention as set out above.

15

EXAMPLES

Example 1 - Amplification and cloning of early open reading frames (ORFs) of HPV6b

20 A clone containing the entire genome of HPV6b in pBR322 (de Villiers, 1981, *J. Virol*, 40:932) was used as the template for separate PCR amplifications of E6, E7, E5a, E5b, E1, E2 and E4 open reading frame (ORF) sequences.

Appropriate restriction enzyme recognition sequences were included in the
25 oligonucleotides used for amplification (Table I; 1-7) to allow sequential assembly of these amplified early gene sequences into a 'polyprotein' sequence as depicted in Figure 1A.

- 16 -

In this scheme, E6 was amplified with oligonucleotides containing a *Sma*I site at the 5' end and *Hind*III, *Nco*I and *Xba*I sites at the 3' end. As well, E4 was amplified with oligonucleotides containing *Xba*I, *Sac*I, *Kpn*I and *Spe*I sites 5' and a *Bgl*II site 3'.

5 These amplified fragments were cloned as *Sma*I/*Xba*I (E6) and *Xba*I/*Bgl*II (E4) (Figure 1B) in the vector pSP70 (Promega Corporation) which had been modified by the removal of an *Eco*RV/*Eco*RI fragment to contain a portion of the pGEM3Zf (Promega Corporation) polylinker - *Hind*II through *Eco*RI. As well, unwanted sites upstream of the *Sma*I site were removed by cleaving with *Sma*I/*Xho*I and insertion of a *Sma*I/*Sal*I/*Xho*I
10 linker to create the vector pSP70 (MOD).

The E6/E4 cassette was able to be removed by cleavage with *Sma*I/*Bgl*II and this was then cloned for expression into the pGEX-STOP vector which produces a non-fusion protein with a C-terminal six-histidine sequence for purification purposes.

15

Using the introduced restriction enzyme recognition sequences, other early ORF sequences were incorporated into the E6/E4 cassette cloned into pSP70 (MOD) and then the newly created cassette cloned as a *Sma*I/*Bgl*II fragment into pGEX-STOP.

20 In this manner polyprotein constructs containing E6/E5a/E4, E6/E7/E4, E6/E7/E5a/E4, E6/E7/E1/E4 and E6/E7/E5a/E1/E4 were assembled. Complete DNA sequence data for the first three constructs is included and sequence data across the junctions of E1 is included for the latter two. DNA sequencing revealed the *Spe*I site was inactivated by a single base change which occurred either during oligonucleotide
25 synthesis, PCR or cloning.

As well the tetrafusion construct of E6/E7/E5a/E4 was cloned for expression into pET23b (Novagen) by firstly subcloning the tetramer as a *Sma*I/*Bgl*II fragment into the *Sma*I/*Bam*HI sites of the vector pRIT2T (AMRAD Pharmacia Biotech). The tetramer was

- 17 -

then removed by restriction with *Sma*I and *Sa*II and cloned into the *Hinc*II/*Xho*I sites of the vector pET23b.

A further construct containing E2 and E5b, but which could also accommodate the
5 addition of E1 and E5a, was created by amplifying E2 with oligonucleotides containing
a *Sma*I site at the 5' end and *Xba*I, *Nco*I, *Kpn*I and *Sac*I, sites at the 3' end (Table 1; 8) and
with E5b amplified using oligonucleotides with an *Xba*I site 5' and *Xho*I, *Bgl*II sites 3'
(Table 1; 9). These amplified fragments were then cloned into pSP70 (MOD) as depicted
in Figure 1C.

Table 1

| Oligonucleotides used for PCR | | |
|-------------------------------|---|--|
| Early gene | Forward | Reverse |
| 1 E6 | 5'GCGCCCCGGGATGGAAAGTGC AAATGCCTC ^{3'} (SEQ ID No. 1) | 5'GCGCTCTAGACCATGGAAGCT TGGGTAACATGTCTTCCATGC ^{3'} (SEQ ID. No.2) |
| 2 E4 | 5'GCGCTCTAGAGAGCTCGGTACC ACTAGTGGAGCACCAAACATTGG GAAG ^{3'} (SEQ ID No. 3) | 5'GCGCAGATCTTAGGCGTAGCT GAACTGTTAC ^{3'} (SEQ ID No. 4) |
| 3 E5a | 5'GCGCCCATGGGAAGTGGTGCCT GTACAAATAGC ^{3'} (SEQ ID No. 5) | 5'GCGCTCTAGATTGCTGTGTGG TAACAATATAG ^{3'} (SEQ ID No. 6) |
| 4 E7 | 5'GCGCAAGCTTCATGGAAGACAT GTTACCCTAAAG ^{3'} (SEQ ID No. 7) | 5'GCGCCCATGGGGTCTTCGGT GCGCAGATGG ^{3'} (SEQ ID No. 8) |
| 5 E1 | 5'GCGCGAGCTCGCGGACGATTCA GGTACAGAAAATG ^{3'} (SEQ ID No. 9) | 5'GCGCGGTACCTAAAGTTCTAA CAACTGTTCTG ^{3'} (SEQ ID No. 10) |
| 6 E2 | 5'GCGCGGTACCGAAGCAATAGCC AAGCGTTTAG ^{3'} (SEQ ID No. 11) | 5'GCGCACTAGTCAATAGGTGCA GTGACATAAATC ^{3'} (SEQ ID No. 12) |
| 7 E5b | 5'GCGCTCTAGACTAACATGTCAAT TTAATGATG ^{3'} (SEQ ID No. 13) | 5'GCGCGAGCTCATTCATATATA TATAATCACC ^{3'} (SEQ ID No. 14) |
| 8 E2 | 5'GCGCCCCGGGATGGAAGCAATA GCCAAGCG ^{3'} (SEQ ID No. 15) | 5'GCGCTCTAGACCATGGGGTAC CGAGCTCCAATAGGTGCAGTG ACATAAATC ^{3'} (SEQ ID No. 16) |
| 9 E5b | 5'GCGCTCTAGACTAACATGTCAAT TTAATGATG ^{3'} (SEQ ID No. 17) | 5'GCGCAGATCTCTCGAGATTCA TATATATATAATCAC ^{3'} (SEQ ID No. 18) |

- 19 -

Example 2 - Expression of different polyprotein constructs

The following constructs in pGEX-STOP were expressed in *E. coli* strain BL21 and protein production was assayed by PAGE followed by Western blotting:

5

- i) E6/E4
- ii) E6/E5a/E4
- iii) E6/E7/E4
- iv) E6/E7/E5a/E4

10

Construct (iv) in pET23b, expressed in *E. coli* strains BL21(DE3)pLysS and AD494(DE3)pLysS (Novagen), was also assayed for protein production by Western blotting and also by Coomassie Blue staining for the latter strain.

15

Cultures of 200mL were grown in Terrific broth (Tartoff and Hobbs, *Focus*, 9: 12, 1987) in the presence of 100 μ g/mL ampicillin (BL21) and 34 μ g/ml chloramphenicol [BL21(DE3)pLysS] and 15 μ g/mL kanamycin [AD494(DE3)pLysS]. At OD₆₀₀ ~ 1 protein expression was induced by the addition of IPTG to 0.4mM. Following induction samples were taken at 1, 2, 3, 4 and 5 hours and in some cases after overnight culture.

20

Figure 2 shows a Western blot result for the E6/E4 construct. This was probed with a polyclonal rabbit anti-E4 antibody (MWE4 - raised to the peptide LGNEHEESNSPLATPCVWPT conjugated to ovalbumin). An immunoreactive band of ~30 kDa was present in the 4 hour-induced sample (lanes 2 & 4, arrow) which was not
25 present in the uninduced sample (lane 3).

The same ~30kDa band can also be seen in the induced sample in Figure 3, lane 3, arrow (lane 2-uninduced) while the E6/E5a/E4 trimer construct of ~ 40kDa was poorly represented after a 4 hour induction period (lane 5, arrow; uninduced sample-lane 4)
30 using the same anti-E4 antibody.

- 20 -

In contrast however, a trimer construct of E6/E7/E4 (~ 41 kDa) could be easily detected after 5 hours induction using an anti-hexahistidine monoclonal antibody (Dianova) [Figure 4, lane 4, arrow; uninduced sample - lane 3].

5 The same trimer construct was again easily visualised after 5 hours induction using the anti-E4 antibody MWE4 (Figure 5, lane TRI, arrow; control sample - lane C) and the tetramer consisting of E6/E7/E5a/E4 (~ 51 kDa) could also be detected (lane TET, arrow). Although this band is weak, it must be noted that a considerable amount of high molecular weight material is also immunoreactive, indicating the tetramer is reasonably
10 well expressed but possibly prone to aggregation.

Figure 6 indicates that an anti-E6 antibody (prepared as described below) was able to detect E6/E7/E4 after 5 hours induction (lane TRI, arrow) but not E6/E7/E5a/E4 (lane TET; lane C - uninduced). However, an anti-E7 antibody (prepared as described below)
15 was able to detect after 5 hours induction both the trimer (Figure 7, lane TRI, arrow; lane C - uninduced) and the tetramer (lane TET, arrow; lane C - uninduced), with the latter again showing indications of aggregation. A monoclonal antibody raised to an E4 peptide also recognised the trimer.

20 The phenomenon of aggregation was clearly apparent when the E6/E7/E5a/E4 tetramer was expressed in the pET23b plasmid in BL21(DE3)pLysS (Figure 8 - a Western blot probed with MWE4). Lanes 2-5 are 1 hour, 2 hour, 3 hour and overnight uninduced samples and lanes 6-9 represent 1 hour, 2 hour, 3 hour and overnight induced samples. After 1 hour induction a band of E6/E7/E5a/E4 can clearly be seen (arrow), but with
25 increased times of induction this seems to decrease and aggregated forms are increased. In contrast, when strain AD494(DE3)pLysS was used to express the tetramer, a substantial signal was obtained at the ~ 50kDa position on a Western blot of the insoluble fraction (Figure 9, arrow) following 2 hours induction, which still persisted at 3 hours. This immunoreactive band was not present in control samples and no protein was detected
30 in the samples from the soluble fractions.

- 21 -

Figure 10 shows the Coomassie stained profile of an identical gel, indicating that the immunoreactive bands present after 2 and 3 hours induction (Figure 9) can clearly be visualised as stained bands (arrow) which are not present in the control samples.

5 Example 3 - DNA sequencing of polyprotein constructs

Polyprotein constructs were sequenced in both directions by the dideoxy method using primers that generated overlapping sequence information. The ¹⁷Sequencing™ Kit (Pharmacia) was used to generate ³⁵S-labelled chain-terminated fragments which were
10 analysed on a Sequi-Gen™ (Biorad) electrophoretic gel apparatus. The DNA and corresponding amino acid sequences for E6/E5a/E4 (CSL690.SEQ), E6/E7/E4 (CSL760.SEQ) and E6/E7/E5a/E4 (CSL673.SEQ) are shown below. (SEQ ID Nos: 19 and 20, 21 and 22, and 23 and 24, respectively).

15 For constructs E6/E7/E1/E4 (CSL 791) and E6/E7/E5a/E1/E4 (CSL 762), which were created from E6/E7/E4 and E6/E7/E5a/E4, respectively, DNA sequence analysis across the junctions of E1 with its neighbours is shown below (SEQ ID Nos. 25 and 26, 27 and 28, and 29 and 30, respectively).

- 22 -

File : CSL690.SEQ

Range : 1 - 11 Mode : Normal

Codon Table : Universal

E6/E5a/E4 - SEQ ID Nos, 19 (DNA) and 20 (amino acid)

| | 9 | 18 | 27 | 36 | 45 | 54 | | | | | | | | | | | | |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 5' | ATG | GAA | AGT | GCA | AAT | GCC | TCC | ACG | TCT | GCA | ACG | ACC | ATA | GAC | CAG | TTG | TGC | AAG |
| | Met | Glu | Ser | Ala | Asn | Ala | Ser | Thr | Ser | Ala | Thr | Thr | Ile | Asp | Gln | Leu | Cys | Lys |
| | 63 | 72 | 81 | 90 | 99 | 108 | | | | | | | | | | | | |
| | ACG | TTT | AAT | CTA | TCT | ATG | CAT | ACG | TTG | CAA | ATT | AAT | TGT | GTG | TTT | TGC | AAG | AAT |
| | Thr | Phe | Asn | Leu | Ser | Met | His | Thr | Leu | Gln | Ile | Asn | Cys | Val | Phe | Cys | Lys | Asn |
| | 117 | 126 | 135 | 144 | 153 | 162 | | | | | | | | | | | | |
| | GCA | CTG | ACC | ACA | GCA | GAG | ATT | TAT | TCA | TAT | GCA | TAT | AAA | CAC | CTA | AAG | GTC | CTG |
| | Ala | Leu | Thr | Thr | Ala | Glu | Ile | Tyr | Ser | Tyr | Ala | Tyr | Lys | His | Leu | Lys | Val | Leu |
| | 171 | 180 | 189 | 198 | 207 | 216 | | | | | | | | | | | | |
| | TTT | CGA | GGC | GGC | TAT | CCA | TAT | GCA | GCC | TGC | GCG | TGC | TGC | CTA | GAA | TTT | CAT | GGA |
| | Phe | Arg | Gly | Gly | Tyr | Pro | Tyr | Ala | Ala | Cys | Ala | Cys | Cys | Leu | Glu | Phe | His | Gly |
| | 225 | 234 | 243 | 252 | 261 | 270 | | | | | | | | | | | | |
| | AAA | ATA | AAC | CAA | TAT | AGA | CAC | TTT | GAT | TAT | GCT | GGA | TAT | GCA | ACA | ACA | GTT | GAA |
| | Lys | Ile | Asn | Gln | Tyr | Arg | His | Phe | Asp | Tyr | Ala | Gly | Tyr | Ala | Thr | Thr | Val | Glu |
| | 279 | 288 | 297 | 306 | 315 | 324 | | | | | | | | | | | | |
| | GAA | GAA | ACT | AAA | CAA | GAC | ATC | TTA | GAC | GTG | CTA | ATT | CGG | TGC | TAC | CTG | TGT | CAC |
| | Glu | Glu | Thr | Lys | Gln | Asp | Ile | Leu | Asp | Val | Leu | Ile | Arg | Cys | Tyr | Leu | Cys | His |
| | 333 | 342 | 351 | 360 | 369 | 378 | | | | | | | | | | | | |
| | AAA | CCG | CTG | TGT | GAA | GTA | GAA | AAG | GTA | AAA | CAT | ATA | CTA | ACC | AAG | GCG | CGG | TTC |
| | Lys | Pro | Leu | Cys | Glu | Val | Glu | Lys | Val | Lys | His | Ile | Leu | Thr | Lys | Ala | Arg | Phe |
| | 387 | 396 | 405 | 414 | 423 | 432 | | | | | | | | | | | | |
| | ATA | AAG | CTA | AAT | TGT | ACG | TGG | AAG | GGT | CGC | TGC | CTA | CAC | TGC | TGG | ACA | ACA | TGC |
| | Ile | Lys | Leu | Asn | Cys | Thr | Trp | Lys | Gly | Arg | Cys | Leu | His | Cys | Trp | Thr | Thr | Cys |
| | 441 | 450 | 459 | 468 | 477 | 486 | | | | | | | | | | | | |
| | ATG | GAA | GAC | ATG | TTA | CCC | AAG | CTT | CCA | TGG | GAA | GTG | GTG | CCT | GTA | CAA | ATA | GCT |
| | Met | Glu | Asp | Met | Leu | Pro | Lys | Leu | Pro | Trp | Glu | Val | Val | Pro | Val | Gln | Ile | Ala |
| | 495 | 504 | 513 | 522 | 531 | 540 | | | | | | | | | | | | |
| | GCA | GGA | ACA | ACC | AGC | ACA | TTC | ATA | CTG | CCT | GTT | ATA | ATT | GCA | TTT | GTT | GTA | TGT |
| | Ala | Gly | Thr | Thr | Ser | Thr | Phe | Ile | Leu | Pro | Val | Ile | Ile | Ala | Phe | Val | Val | Cys |
| | 549 | 558 | 567 | 576 | 585 | 594 | | | | | | | | | | | | |

- 23 -

```

TTT GTT AGC ATC ATA CTT ATT GTA TGG ATA TCT GAG TTT ATT GTG TAC ACA TCT
---
Phe Val Ser Ile Ile Leu Ile Val Trp Ile Ser Glu Phe Ile Val Tyr Thr Ser

        603          612          621          630          639          648

GTG CTA GTA CTA ACA CTG CTT TTA TAT TTA CTA TTG TGG CTG CTA TTA ACA ACC
---
Val Leu Val Leu Thr Leu Leu Leu Tyr Leu Leu Leu Trp Leu Leu Leu Thr Thr

        657          666          675          684          693          702

CCC TTG CAA TTT TTC CTA CTA ACT CTA CTT GTG TGT TAC TGT CCC GCA TTG TAT
---
Pro Leu Gln Phe Phe Leu Leu Thr Leu Leu Val Cys Tyr Cys Pro Ala Leu Tyr

        711          720          729          738          747          756

ATA CAC TAC TAT ATT GTT ACC ACA CAG CAA TCT AGA GAG CTC GGT ACC ACT AAT
---
Ile His Tyr Tyr Ile Val Thr Thr Gln Gln Ser Arg Glu Leu Gly Thr Thr Asn

        765          774          783          792          801          810

GGA GCA CCA AAC ATT GGG AAG TAT GTT ATG GCA GCA CAG TTA TAT GTT CTC CTG
---
Gly Ala Pro Asn Ile Gly Lys Tyr Val Met Ala Ala Gln Leu Tyr Val Leu Leu

        819          828          837          846          855          864

CAT CTG TAT CTA GCA CTA CAC AAG AAG TAT CCA TTC CTG AAT CTA CTA CAT ACA
---
His Leu Tyr Leu Ala Leu His Lys Lys Tyr Pro Phe Leu Asn Leu Leu His Thr

        873          882          891          900          909          918

CCC CCG CAC AGA CCT CCA CCC TTG TGT CCT CAA GCA CCA AGG AAG ACG CAG TGC
---
Pro Pro His Arg Pro Pro Pro Leu Cys Pro Gln Ala Pro Arg Lys Thr Gln Cys

        927          936          945          954          963          972

AAA CGC CGC CTA GGA AAC GAG CAC GAG GAG TCC AAC AGT CCC CTT GCA ACG CCT
---
Lys Arg Arg Leu Gly Asn Glu His Glu Glu Ser Asn Ser Pro Leu Ala Thr Pro

        981          990          999          1008          1017          1026

TGT GTG TGG CCC ACA TTG GAC CCG TGG ACA GTG GAA ACC ACA ACC TCA TCA CTA
---
Cys Val Trp Pro Thr Leu Asp Pro Trp Thr Val Glu Thr Thr Thr Ser Ser Leu

        1035          1044          1053          1062          1071          1080

ACA ATC ACG ACC AGC ACC AAA GAC GGA ACA ACA GTA ACA GTT CAG CTA CGC CTA
---
Thr Ile Thr Thr Ser Thr Lys Asp Gly Thr Thr Val Thr Val Gln Leu Arg Leu

        1089          1098          1107

AGA TCT CAT CAC CAT CAC CAT CAC TAA 3'
---
Arg Ser His His His His His His ***

```

- 24 -

File : CSL760.SEQ

Range : 1 - 1128 Mode : Normal

Codon Table : Universal

E6/E7/E4 - SEQ ID Nos. 21 (DNA) and 22 (amino acid)

| | 9 | 18 | 27 | 36 | 45 | 54 | | | | | | | | | | | | |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 5' | ATG | GAA | AGT | GCA | AAT | GCC | TCC | ACG | TCT | GCA | ACG | ACC | ATA | GAC | CAG | TTG | TGC | AAG |
| | Met | Glu | Ser | Ala | Asn | Ala | Ser | Thr | Ser | Ala | Thr | Thr | Ile | Asp | Gln | Leu | Cys | Lys |
| | 63 | 72 | 81 | 90 | 99 | 108 | | | | | | | | | | | | |
| | ACG | TTT | AAT | CTA | TCT | ATG | CAT | ACG | TTG | CAA | ATT | AAT | TGT | GTG | TTT | TGC | AAG | AAT |
| | Thr | Phe | Asn | Leu | Ser | Met | His | Thr | Leu | Gln | Ile | Asn | Cys | Val | Phe | Cys | Lys | Asn |
| | 117 | 126 | 135 | 144 | 153 | 162 | | | | | | | | | | | | |
| | GCA | CTG | ACC | ACA | GCA | GAG | ATT | TAT | TCA | TAT | GCA | TAT | AAA | CAC | CTA | AAG | GTC | CTG |
| | Ala | Leu | Thr | Thr | Ala | Glu | Ile | Tyr | Ser | Tyr | Ala | Tyr | Lys | His | Leu | Lys | Val | Leu |
| | 171 | 180 | 189 | 198 | 207 | 216 | | | | | | | | | | | | |
| | TTT | CGA | GGC | GGC | TAT | CCA | TAT | GCA | GCC | TGC | GCG | TGC | TGC | CTA | GAA | TTT | CAT | GGA |
| | Phe | Arg | Gly | Gly | Tyr | Pro | Tyr | Ala | Ala | Cys | Ala | Cys | Cys | Leu | Glu | Phe | His | Gly |
| | 225 | 234 | 243 | 252 | 261 | 270 | | | | | | | | | | | | |
| | AAA | ATA | AAC | CAA | TAT | AGA | CAC | TTT | GAT | TAT | GCT | GGA | TAT | GCA | ACA | ACA | GTT | GAA |
| | Lys | Ile | Asn | Gln | Tyr | Arg | His | Phe | Asp | Tyr | Ala | Gly | Tyr | Ala | Thr | Thr | Val | Glu |
| | 279 | 288 | 297 | 306 | 315 | 324 | | | | | | | | | | | | |
| | GAA | GAA | ACT | AAA | CAA | GAC | ATC | TTA | GAC | GTG | CTA | ATT | CGG | TGC | TAC | CTG | TGT | CAC |
| | Glu | Glu | Thr | Lys | Gln | Asp | Ile | Leu | Asp | Val | Leu | Ile | Arg | Cys | Tyr | Leu | Cys | His |
| | 333 | 342 | 351 | 360 | 369 | 378 | | | | | | | | | | | | |
| | AAA | CCG | CTG | TGT | GAA | GTA | GAA | AAG | GTA | AAA | CAT | ATA | CTA | ACC | AAG | GCG | CGG | TTC |
| | Lys | Pro | Leu | Cys | Glu | Val | Glu | Lys | Val | Lys | His | Ile | Leu | Thr | Lys | Ala | Arg | Phe |
| | 387 | 396 | 405 | 414 | 423 | 432 | | | | | | | | | | | | |
| | ATA | AAG | CTA | AAT | TGT | ACG | TGG | AAG | GGT | CGC | TGC | CTA | CAC | TGC | TGG | ACA | ACA | TGC |
| | Ile | Lys | Leu | Asn | Cys | Thr | Trp | Lys | Gly | Arg | Cys | Leu | His | Cys | Trp | Thr | Thr | Cys |
| | 441 | 450 | 459 | 468 | 477 | 486 | | | | | | | | | | | | |
| | ATG | GAA | GAC | ATG | TTA | CCC | AAG | CTT | CAT | GGA | AGA | CAT | GTT | ACC | CTA | AAG | GAT | ATT |
| | Met | Glu | Asp | Met | Leu | Pro | Lys | Leu | His | Gly | Arg | His | Val | Thr | Leu | Lys | Asp | Ile |
| | 495 | 504 | 513 | 522 | 531 | 540 | | | | | | | | | | | | |
| | GTA | TTA | GAC | CTG | CAA | CCT | CCA | GAC | CCT | GTA | GGG | TTA | CAT | TGC | TAT | GAG | CAA | TTA |
| | Val | Leu | Asp | Leu | Gln | Pro | Pro | Asp | Pro | Val | Gly | Leu | His | Cys | Tyr | Glu | Gln | Leu |
| | 549 | 558 | 567 | 576 | 585 | 594 | | | | | | | | | | | | |

- 25 -

```

GTA GAC AGC TCA GAA GAT GAG GTG GAC GAA GTG GAC GGA CAA GAT TCA CAA CCT
---
Val Asp Ser Ser Glu Asp Glu Val Asp Glu Val Asp Gly Gln Asp Ser Gln Pro
      603          612          621          630          639          648

TTA AAA CAA CAT TTC CAA ATA GTG ACC TGT TGC TGT GGA TGT GAC AGC AAC GTT
---
Leu Lys Gln His Phe Gln Ile Val Thr Cys Cys Cys Gly Cys Asp Ser Asn Val
      657          666          675          684          693          702

CGA CTG GTT GTG CAG TGT ACA GAA ACA GAC ATC AGA GAA GTG CAA CAG CTT CTG
---
Arg Leu Val Val Gln Cys Thr Glu Thr Asp Ile Arg Glu Val Gln Gln Leu Leu
      711          720          729          738          747          756

TTG GGA ACA CTA AAC ATA GTG TGT CCC ATC TGC GCA CCG AAG ACC CCA TGG TCT
---
Leu Gly Thr Leu Asn Ile Val Cys Pro Ile Cys Ala Pro Lys Thr Pro Trp Ser
      765          774          783          792          801          810

AGA GAG CTC GGT ACC ACT AAT GGA GCA CCA AAC ATT GGG AAG TAT GTT ATG GCA
---
Arg Glu Leu Gly Thr Thr Asn Gly Ala Pro Asn Ile Gly Lys Tyr Val Met Ala
      819          828          837          846          855          864

GCA CAG TTA TAT GTT CTC CTG CAT CTG TAT CTA GCA CTA CAC AAG AAG TAT CCA
---
Ala Gln Leu Tyr Val Leu Leu His Leu Tyr Leu Ala Leu His Lys Lys Tyr Pro
      873          882          891          900          909          918

TTC CTG AAT CTA CTA CAT ACA CCC CCG CAC AGA CCT CCA CCC TTG TGT CCT CAA
---
Phe Leu Asn Leu Leu His Thr Pro Pro His Arg Pro Pro Pro Leu Cys Pro Gln
      927          936          945          954          963          972

GCA CCA AGG AAG ACG CAG TGC AAA CGC CGC CTA GGA AAC GAG CAC GAG GAG TCC
---
Ala Pro Arg Lys Thr Gln Cys Lys Arg Arg Leu Gly Asn Glu His Glu Glu Ser
      981          990          999          1008          1017          1026

AAC AGT CCC CTT GCA ACG CCT TGT GTG TGG CCC ACA TTG GAC CCG TGG ACA GTG
---
Asn Ser Pro Leu Ala Thr Pro Cys Val Trp Pro Thr Leu Asp Pro Trp Thr Val
      1035          1044          1053          1062          1071          1080

GAA ACC ACA ACC TCA TCA CTA ACA ATC ACG ACC AGC ACC AAA GAC GGA ACA ACA
---
Glu Thr Thr Thr Ser Ser Leu Thr Ile Thr Thr Ser Thr Lys Asp Gly Thr Thr
      1089          1098          1107          1116          1125

GTA ACA GTT CAG CTA CGC CTA AGA TCT CAT CAC CAT CAC CAT CAC TAA 3'
---
Val Thr Val Gln Leu Arg Leu Arg Ser His His His His His His His ***

```

- 26 -

File : CSL673.DNA

Range : 1 - 1300 Mode : Normal

Codon Table : Universal

E6/E7/E5a/E4 - SEQ ID Nos. 23 (DNA) and 24 (amino acid)

| | 9 | 18 | 27 | 36 | 45 | 54 |
|----|---|-----|-----|-----|-----|-----|
| 5' | ATG GAA AGT GCA AAT GCC TCC ACG TCT GCA ACG ACC ATA GAC CAG TTG TGC AAG | | | | | |
| | Met Glu Ser Ala Asn Ala Ser Thr Ser Ala Thr Thr Ile Asp Gln Leu Cys Lys | | | | | |
| | 63 | 72 | 81 | 90 | 99 | 108 |
| | ACG TTT AAT CTA TCT ATG CAT ACG TTG CAA ATT AAT TGT GTG TTT TGC AAG AAT | | | | | |
| | Thr Phe Asn Leu Ser Met His Thr Leu Gln Ile Asn Cys Val Phe Cys Lys Asn | | | | | |
| | 117 | 126 | 135 | 144 | 153 | 162 |
| | GCA CTG ACC ACA GCA GAG ATT TAT TCA TAT GCA TAT AAA CAC CTA AAG GTC CTG | | | | | |
| | Ala Leu Thr Thr Ala Glu Ile Tyr Ser Tyr Ala Tyr Lys His Leu Lys Val Leu | | | | | |
| | 171 | 180 | 189 | 198 | 207 | 216 |
| | TTT CGA GGC GGC TAT CCA TAT GCA GCC TGC GCG TGC TGC CTA GAA TTT CAT GGA | | | | | |
| | Phe Arg Gly Gly Tyr Pro Tyr Ala Ala Cys Ala Cys Cys Leu Glu Phe His Gly | | | | | |
| | 225 | 234 | 243 | 252 | 261 | 270 |
| | AAA ATA AAC CAA TAT AGA CAC TTT GAT TAT GCT GGA TAT GCA ACA ACA GTT GAA | | | | | |
| | Lys Ile Asn Gln Tyr Arg His Phe Asp Tyr Ala Gly Tyr Ala Thr Thr Val Glu | | | | | |
| | 279 | 288 | 297 | 306 | 315 | 324 |
| | GAA GAA ACT AAA CAA GAC ATC TTA GAC GTG CTA ATT CGG TGC TAC CTG TGT CAC | | | | | |
| | Glu Glu Thr Lys Gln Asp Ile Leu Asp Val Leu Ile Arg Cys Tyr Leu Cys His | | | | | |
| | 333 | 342 | 351 | 360 | 369 | 378 |
| | AAA CCG CTG TGT GAA GTA GAA AAG GTA AAA CAT ATA CTA ACC AAG GCG CGG TTC | | | | | |
| | Lys Pro Leu Cys Glu Val Glu Lys Val Lys His Ile Leu Thr Lys Ala Arg Phe | | | | | |
| | 387 | 396 | 405 | 414 | 423 | 432 |
| | ATA AAG CTA AAT TGT ACG TGG AAG GGT CGC TGC CTA CAC TGC TGG ACA ACA TGC | | | | | |
| | Ile Lys Leu Asn Cys Thr Trp Lys Gly Arg Cys Leu His Cys Trp Thr Thr Cys | | | | | |
| | 441 | 450 | 459 | 468 | 477 | 486 |
| | ATG GAA GAC ATG TTA CCC AAG CTT CAT GGA AGA CAT GTT ACC CTA AAG GAT ATT | | | | | |
| | Met Glu Asp Met Leu Pro Lys Leu His Gly Arg His Val Thr Leu Lys Asp Ile | | | | | |
| | 495 | 504 | 513 | 522 | 531 | 540 |
| | GTA TTA GAC CTG CAA CCT CCA GAC CCT GTA GGG TTA CAT TGC TAT GAG CAA TTA | | | | | |
| | Val Leu Asp Leu Gln Pro Pro Asp Pro Val Gly Leu His Cys Tyr Glu Gln Leu | | | | | |
| | 549 | 558 | 567 | 576 | 585 | 594 |

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| | | | | | | | | | | | | | | | | | |
|------|-----|-----|-----|------|-----|-----|-----|------|-----|-----|-----|------|-----|-----|------|-----|------|
| GTA | GAC | AGC | TCA | GAA | GAT | GAG | GTG | GAC | GAA | GTG | GAC | GGA | CAA | GAT | TCA | CAA | CCT |
| Val | Asp | Ser | Ser | Glu | Asp | Glu | Val | Asp | Glu | Val | Asp | Gly | Gln | Asp | Ser | Gln | Pro |
| 603 | | | | 612 | | | | 621 | | | | 630 | | | 639 | | 648 |
| TTA | AAA | CAA | CAT | TTC | CAA | ATA | GTG | ACC | TGT | TGC | TGT | GGA | TGT | GAC | AGC | AAC | GTT |
| Leu | Lys | Gln | His | Phe | Gln | Ile | Val | Thr | Cys | Cys | Cys | Gly | Cys | Asp | Ser | Asn | Val |
| 657 | | | | 666 | | | | 675 | | | | 684 | | | 693 | | 702 |
| CGA | CTG | GTT | GTG | CAG | TGT | ACA | GAA | ACA | GAC | ATC | AGA | GAA | GTG | CAA | CAG | CTT | CTG |
| Arg | Leu | Val | Val | Gln | Cys | Thr | Glu | Thr | Asp | Ile | Arg | Glu | Val | Gln | Gln | Leu | Leu |
| 711 | | | | 720 | | | | 729 | | | | 738 | | | 747 | | 756 |
| TTG | GGA | ACA | CTA | AAC | ATA | GTG | TGT | CCC | ATC | TGC | GCA | CCG | AAG | ACC | CCA | TGG | GAA |
| Leu | Gly | Thr | Leu | Asn | Ile | Val | Cys | Pro | Ile | Cys | Ala | Pro | Lys | Thr | Pro | Trp | Glu |
| 765 | | | | 774 | | | | 783 | | | | 792 | | | 801 | | 810 |
| GTG | GTG | CCT | GTA | CAA | ATA | GCT | GCA | GGA | ACA | ACC | AGC | ACA | TTC | ATA | CTG | CCT | GTT |
| Val | Val | Pro | Val | Gln | Ile | Ala | Ala | Gly | Thr | Thr | Ser | Thr | Phe | Ile | Leu | Pro | Val |
| 819 | | | | 828 | | | | 837 | | | | 846 | | | 855 | | 864 |
| ATA | ATT | GCA | TTT | GTT | GTA | TGT | TTT | GTT | AGC | ATC | ATA | CTT | ATT | GTA | TGG | ATA | TCT |
| Ile | Ile | Ala | Phe | Val | Val | Cys | Phe | Val | Ser | Ile | Ile | Leu | Ile | Val | Trp | Ile | Ser |
| 873 | | | | 882 | | | | 891 | | | | 900 | | | 909 | | 918 |
| GAG | TTT | ATT | GTG | TAC | ACA | TCT | GTG | CTA | GTA | CTA | ACA | CTG | CTT | TTA | TAT | TTA | CTA |
| Glu | Phe | Ile | Val | Tyr | Thr | Ser | Val | Leu | Val | Leu | Thr | Leu | Leu | Leu | Tyr | Leu | Leu |
| 927 | | | | 936 | | | | 945 | | | | 954 | | | 963 | | 972 |
| TTG | TGG | CTG | CTA | TTA | ACA | ACC | CCC | TTG | CAA | TTT | TTC | CTA | CTA | ACT | CTA | CTT | GTG |
| Leu | Trp | Leu | Leu | Leu | Thr | Thr | Pro | Leu | Gln | Phe | Phe | Leu | Leu | Thr | Leu | Leu | Val |
| 981 | | | | 990 | | | | 999 | | | | 1008 | | | 1017 | | 1026 |
| TGT | TAC | TGT | CCC | GCA | TTG | TAT | ATA | CAC | TAC | TAT | ATT | GTT | ACC | ACA | CAG | CAA | TCT |
| Cys | Tyr | Cys | Pro | Ala | Leu | Tyr | Ile | His | Tyr | Tyr | Ile | Val | Thr | Thr | Gln | Gln | Ser |
| 1035 | | | | 1044 | | | | 1053 | | | | 1062 | | | 1071 | | 1080 |
| AGA | GAG | CTC | GGT | ACC | ACT | AAT | GGA | GCA | CCA | AAC | ATT | GGG | AAG | TAT | GTT | ATG | GCA |
| Arg | Glu | Leu | Gly | Thr | Thr | Asn | Gly | Ala | Pro | Asn | Ile | Gly | Lys | Tyr | Val | Met | Ala |
| 1089 | | | | 1098 | | | | 1107 | | | | 1116 | | | 1125 | | 1134 |
| GCA | CAG | TTA | TAT | GTT | CTC | CTG | CAT | CTG | TAT | CTA | GCA | CTA | CAC | AAG | AAG | TAT | CCA |
| Ala | Gln | Leu | Tyr | Val | Leu | Leu | His | Leu | Tyr | Leu | Ala | Leu | His | Lys | Lys | Tyr | Pro |
| 1143 | | | | 1152 | | | | 1161 | | | | 1170 | | | 1179 | | 1188 |

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TTC CTG AAT CTA CTA CAT ACA CCC CCG CAC AGA CCT CCA CCC TTG TGT CCT CAA
---
Phe Leu Asn Leu Leu His Thr Pro Pro His Arg Pro Pro Pro Leu Cys Pro Gln

      1197      1206      1215      1224      1233      1242

GCA CCA AGG AAG ACG CAG TGC AAA CGC CGC CTA GGA AAC GAG CAC GAG GAG TCC
---
Ala Pro Arg Lys Thr Gln Cys Lys Arg Arg Leu Gly Asn Glu His Glu Glu Ser

      1251      1260      1269      1278      1287      1296

AAC AGT CCC CTT GCA ACG CCT TGT GTG TGG CCC ACA TTG GAC CCG TGG ACA GTG
---
Asn Ser Pro Leu Ala Thr Pro Cys Val Trp Pro Thr Leu Asp Pro Trp Thr Val

      1305      1314      1323      1332      1341      1350

GAA ACC ACA ACC TCA TCA CTA ACA ATC ACG ACC AGC ACC AAA GAC GGA ACA ACA
---
Glu Thr Thr Thr Ser Ser Leu Thr Ile Thr Thr Ser Thr Lys Asp Gly Thr Thr

      1359      1368      1377      1386      1395

GTA ACA GTT CAG CTA CGC CTA AGA TCT CAT CAC CAT CAC CAT CAC TAA 3'
---
Val Thr Val Gln Leu Arg Leu Arg Ser His His His His His His ***

```

Junction of E1 and E4 ORFs for CSL791 and CSL762

SEQ ID Nos. 25 (DNA) and 26 (amino acid)

5' GAG GAA GAT GGA AGC AAT AGC CAA GCG TTT AGA TGC GTG CCA GGA ACA GTT GTT AGA ACT TTA GGT ACC ACT AAT GGA GCA CCA AAC ATT GGG AAG TAT GTT ATG GCA 3'
 Glu Glu Asp Gly Ser Asn Ser Gln Ala Phe Arg Cys Val Pro Gly Thr Val Val Arg Thr Leu Gly Thr Thr Asn Gly Ala Pro Asn Ile Gly Lys Tyr Val Met Ala

Modified
 Xba1 Spe1

E1 E4

Junction of E5a and E1 for CSL762

SEQ ID Nos. 27 (DNA) and 28 (amino acid)

5' TGT CCC GCA TTG TAT ATA CAC TAC TAT ATT GTT ACC ACA CAG CAA TCT AGA GAG CTC GCG GAC GAT TCA GGT ACA GAA AAT GAG GGG TCT GGG TGT ACA GGA 3'
 Cys Pro Ala Leu Tyr Ile His Tyr Tyr Ile Val Thr Thr Gln Ser Arg Glu Leu Ala Asp Asp Ser Gly Thr Glu Asn Glu Gly Ser Gly Cys Thr Gly

Xba1 Sac1

E5a E1

Junction of E7 and E1 for CSL791

SEQ ID Nos. 29 (DNA) and 30 (amino acid)

5' TTG GGA ACA CTA AAC ATA GTG TGT CCC ATC TGC GCA CCG AAG ACC CCA TGG TCT AGA GAG CTC GCG GAC GAT TCA GGT ACA GAA AAT GAG GGG TCT GGG TGT ACA GGA 3'
 Leu Gly Thr Leu Asn Ile Val Cys Pro Ile Cys Ala Pro Lys Thr Thr Trp Ser Arg Glu Leu Ala Asp Asp Ser Gly Thr Glu Asn Glu Gly Ser Gly Cys Thr Gly

Nco1 Xba1 Sac1

E7 E1

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Example 4 - Preparation of antibodies to HPV6b early ORF protein products

The following peptides corresponding to portions of the sequence of the relevant E proteins, were synthesised and conjugated to diphtheria toxoid:

5

E6 dip. tox-C-QYRHFDYAQYATTVEETKQDILD
E7 MHGRHVTLKDIVLDLQPPD-C-dip. tox

For the E6 peptide two rabbits (following pre-bleeding) were each inoculated with approximately 54µg peptide/104µg diphtheria toxoid in Freund's complete adjuvant followed at 3-weekly intervals by a similar dose of peptide conjugate in Freund's incomplete adjuvant. Bleeds were taken one week after the second dose and one week following the third dose. The same regime was used for the E7 peptide using 45µg peptide/103 µg diphtheria toxoid.

15

Serum derived from the bleeds were tested for specific antibody in a solid phase EIA against biotin-conjugated peptide which had been bound to plates coated with strepavidin.

20 Example 5 - Purification of polyprotein E6/E7/E4

The trimer polyprotein E6/E7/E4 was expressed in *E. coli* BL21 cells by induction of cells at OD₆₀₀ ~ 1 using 0.4mM IPTG. The cells were harvested by centrifugation (4,000g, 20 minutes), resuspended in 30mM Tris pH8.0, disrupted by sonication (MSE, 25 amplitude 18µm, 4 x 30 seconds) and inclusion bodies pelleted by centrifugation (12,000g, 30 minutes). The pellet containing the trimer was solubilized in 8M Urea, 30mM Tris pH8.0 for 16 hours in the presence of protease inhibitors (Boehringer Cat. No. 1697498) and then centrifuged at 12,000g for 30 minutes and the supernatant collected. To this, Tris-(2-carboxyethyl)phosphine (TCEP) [Pierce] was added to 1.2mM final 30 concentration. The supernatant was applied to Q-sepharose HP (Pharmacia) and the

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column washed with one column volume of 8M Urea, 1.2mM TCEP, 30 mM Tris pH8.0. Fractions were then eluted using a gradient containing 0 to 1M NaCl in 10 column volumes of the washing buffer. The fractions obtained were examined by Western blot from 4 to 20% SDS-PAGE probed with the anti-E4 antibody MWE4.

5

Figure 11 shows a Western blot of material obtained from Q-sepharose. An immunoreactive band of ~ 41kDa is evident in supernatant material from the urea solubilisation lane 3, and in fractions corresponding to 120 to 150 mM NaCl (lanes 8 and 9, arrow).

10

Supernatant from the urea solubilisation was also applied to a column containing Chelating Sepharose Fast Flow (Pharmacia) to take advantage of the C-terminal six histidine sequence. Relatively poor binding of the trimer to the Nickel column was observed under the conditions described. The trimer was eluted from the column using a 0 to 500 mM imidazole gradient.

Example 6

In a further example of the present invention, a DNA sequence coding for a single polyprotein (Fig. 12) is formed by fusion of DNA fragments encoding HPV-6 early ORF proteins wherein the order of the ORFs is E2, E4, E5a, E5b, E6, E7 and E1.

The DNA sequences encoding the early ORF proteins are amplified individually by PCR using HPV-6 genomic DNA using the primers set out in Table 2.

25

Table 2

| Gene | Oligonucleotides | |
|------|------------------|--|
| E2 | (a) | 5'-GTG TGT GAG CTC ATG GAA GCA ATA GCC AAG-3' (SEQ ID No. 31) and |
| | (b) | 5'-GTG TGT GTC GAC CAA TAG GTG CAG TGA CAT-3' (SEQ ID No. 32) |
| E4 | (c) | 5'-GTG TGT GTC GAC ATG GGA GCA CCA AAC ATT-3' (SEQ ID No. 33) and |
| | (d) | 5'-GTG TGT AGA TCT TAG GCG TAG CTG AAC TGT-3' (SEQ ID No. 34) |
| E5a | (e) | 5'-GTG TGT AGA TCT ATG GAA GTG GTG CCT GTA-3' (SEQ ID No. 35) and |
| | (f) | 5'-GTG TGT CTT AAG TTG CTG TGT GGT AAC AAT-3' (SEQ ID No. 36) |
| E5b | (g) | 5'-GTG TGT CTT AAG ATG ATG CTA ACA TGT CAA-3' (SEQ ID No. 37) and |
| | (h) | 5'-GTG TGT CCG CGG ATT CAT ATA TAT ATA ATC-3' (SEQ ID No. 38) |
| E6 | (i) | 5'-GTG TGT CCG CGG ATG GAA AGT GCA AAT GCC-3' (SEQ ID No. 39) and |
| | (j) | 5'-GTG TGT GCT AGC GGG TAA CAT GTC TTC CTA-3' (SEQ ID No. 40) |
| E7 | (k) | 5'-GTG TGT GCT AGC ATG CAT GGA AGA CAT GTT-3' (SEQ ID No. 41) and |
| | (l) | 5'-GTG TGT CGA TCG GGT CTT CGG TGC GCA GAT-3' (SEQ ID No. 42) |
| E1 | (m) | 5'-GTG TGT CGA TCG ATG GCG GAC GAT TCA GGT-3' (SEQ ID No. 43) and |
| | (n) | 5'-GTG TGT GGT ACC TCA TAA AGT TCT AAC AAC-3' (SEQ ID No. 44) |

The primers are synthesised to incorporate artificial restriction enzyme sites at the 5' and 3' termini of the amplification products. These restriction enzyme sites are used to facilitate the fusion of PCR products encoding the appropriate early ORF proteins in the desired order and in the correct translational frame. The restriction enzyme sites are also used to aid the cloning of the PCR products into the expression vector pTrcHisA. When cloned into this vector, the polyprotein construct is expressed as an N-terminal

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hexaHis fusion. The nucleotide sequence and deduced amino acid sequence of this fusion are shown below (SEQ ID Nos. 45 and 46, respectively).

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INFORMATION FOR HEXAHIS-POLYPROTEIN FUSION SEQUENCE:

- (I) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 4770 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: HUMAN PAPILLOMAVIRUS TYPE 6
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION:1..4761
 - (D) OTHER INFORMATION:/codon_start= 1
/product= "HPV-6 Polyprotein"
- (ix) FEATURE:
 - (A) NAME/KEY: misc_RNA
 - (B) LOCATION:1..108
 - (D) OTHER INFORMATION:/function= "Tag used for protein purification"
/product= "hexaHis leader sequence from pTrcHisA"
- (ix) FEATURE:
 - (A) NAME/KEY: misc feature
 - (B) LOCATION:109..114
 - (D) OTHER INFORMATION:/label= SacI
- (ix) FEATURE:
 - (A) NAME/KEY: mRNA
 - (B) LOCATION:115..1218
 - (D) OTHER INFORMATION:/gene= "HPV-6 E2"
- (ix) FEATURE:
 - (A) NAME/KEY: misc feature
 - (B) LOCATION:1219..1224
 - (D) OTHER INFORMATION:/label= SalI
- (ix) FEATURE:
 - (A) NAME/KEY: mRNA
 - (B) LOCATION:1225..1551
 - (D) OTHER INFORMATION:/gene= "HPV-6 E4"
- (ix) FEATURE:
 - (A) NAME/KEY: misc feature
 - (B) LOCATION:1552..1557
 - (D) OTHER INFORMATION:/label= BglII
- (ix) FEATURE:
 - (A) NAME/KEY: mRNA
 - (B) LOCATION:1558..1830
 - (D) OTHER INFORMATION:/gene= "HPV-6 E5a"
- (ix) FEATURE:
 - (A) NAME/KEY: misc feature
 - (B) LOCATION:1831..1836
 - (D) OTHER INFORMATION:/label= BfrI
- (ix) FEATURE:
 - (A) NAME/KEY: mRNA
 - (B) LOCATION:1837..2052
 - (D) OTHER INFORMATION:/gene= "HPV-6 E5b"

- (ix) FEATURE:
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 (B) LOCATION:2053..2058
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- (ix) FEATURE:
 (A) NAME/KEY: mRNA
 (B) LOCATION:2059..2508
 (D) OTHER INFORMATION:/gene= "HPV-6 E6"
- (ix) FEATURE:
 (A) NAME/KEY: misc_feature
 (B) LOCATION:2509..2514
 (D) OTHER INFORMATION:/label= NheI
- (ix) FEATURE:
 (A) NAME/KEY: mRNA
 (B) LOCATION:2515..2808
 (D) OTHER INFORMATION:/gene= "HPV-6 E7"
- (ix) FEATURE:
 (A) NAME/KEY: misc_feature
 (B) LOCATION:2809..2814
 (D) OTHER INFORMATION:/label= PvuI
- (ix) FEATURE:
 (A) NAME/KEY: mRNA
 (B) LOCATION:2815..4764
 (D) OTHER INFORMATION:/gene= "HPV-6 E1"
- (ix) FEATURE:
 (A) NAME/KEY: misc_feature
 (B) LOCATION:4765..4770
 (D) OTHER INFORMATION:/label= KpnI

| | |
|---|-----|
| ATG GGG GGT TCT CAT CAT CAT CAT CAT GGT ATG GCT AGC ATG ACT | 48 |
| Met Gly Gly Ser His His His His His Gly Met Ala Ser Met Thr | |
| 1 5 10 15 | |
| GGT GGA CAG CAA ATG GGT CGG GAT CTG TAC GAC GAT GAC GAT AAG GAT | 96 |
| Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp | |
| 20 25 30 | |
| CGA TGG GGA TCC GAG CTC ATG GAA GCA ATA GCC AAG CGT TTA GAT GCG | 144 |
| Arg Trp Gly Ser Glu Leu Met Glu Ala Ile Ala Lys Arg Leu Asp Ala | |
| 35 40 45 | |
| TGC CAG GAA CAG TTG TTA GAA CTT TAT GAA GAA AAC AGT ACT GAC CTA | 192 |
| Cys Gln Glu Gln Leu Leu Glu Leu Tyr Glu Glu Asn Ser Thr Asp Leu | |
| 50 55 60 | |
| CAC AAA CAT GTA TTG CAT TGG AAA TGC ATG AGA CAT GAA AGT GTA TTA | 240 |
| His Lys His Val Leu His Trp Lys Cys Met Arg His Glu Ser Val Leu | |
| 65 70 75 80 | |
| TTA TAT AAA GCA AAA CAA ATG GGC CTA AGC CAC ATA GGA ATG CAA GTA | 288 |
| Leu Tyr Lys Ala Lys Gln Met Gly Leu Ser His Ile Gly Met Gln Val | |
| 85 90 95 | |
| GTG CCA CCA TTA AAG GTG TCC GAA GCA AAA GGA CAT AAT GCC ATT GAA | 336 |
| Val Pro Pro Leu Lys Val Ser Glu Ala Lys Gly His Asn Ala Ile Glu | |
| 100 105 110 | |
| ATG CAA ATG CAT TTA GAA TCA TTA TTA AGG ACT GAG TAT AGT ATG GAA | 384 |
| Met Gln Met His Leu Glu Ser Leu Leu Arg Thr Glu Tyr Ser Met Glu | |
| 115 120 125 | |
| CCG TGG ACA TTA CAA GAA ACA AGT TAT GAA ATG TGG CAA ACA CCA CCT | 432 |
| Pro Trp Thr Leu Gln Glu Thr Ser Tyr Glu Met Trp Gln Thr Pro Pro | |
| 130 135 140 | |
| AAA CGC TGT TTT AAA AAA CGG GGC AAA ACT GTA GAA GTT AAA TTT GAT | 480 |
| Lys Arg Cys Phe Lys Lys Arg Gly Lys Thr Val Glu Val Lys Phe Asp | |

| | | | | |
|--|-----|-----|-----|------|
| 145 | 150 | 155 | 160 | |
| GGC TGT GCA AAC AAT ACA ATG GAT TAT GTG GTA TGG ACA GAT GTG TAT Gly Cys Ala Asn Asn Thr Met Asp Tyr Val Val Trp Thr Asp Val Tyr | 165 | 170 | 175 | 528 |
| GTG CAG GAC AAT GAC ACC TGG GTA AAG GTG CAT AGT ATG GTA GAT GCT Val Gln Asp Asn Asp Thr Trp Val Lys Val His Ser Met Val Asp Ala | 180 | 185 | 190 | 576 |
| AAG GGT ATA TAT TAC ACA TGT GGA CAA TTT AAA ACA TAT TAT GTA AAC Lys Gly Ile Tyr Tyr Thr Cys Gly Gln Phe Lys Thr Tyr Tyr Val Asn | 195 | 200 | 205 | 624 |
| TTT GTA AAA GAG GCA GAA AAG TAT GGG AGC ACC AAA CAT TGG GAA GTA Phe Val Lys Glu Ala Glu Lys Tyr Gly Ser Thr Lys His Trp Glu Val | 210 | 215 | 220 | 672 |
| TGT TAT GGC AGC ACA GTT ATA TGT TCT CCT GCA TCT GTA TCT AGC ACT Cys Tyr Gly Ser Thr Val Ile Cys Ser Pro Ala Ser Val Ser Ser Thr | 225 | 230 | 235 | 720 |
| ACA CAA GAA GTA TCC ATT CCT GAA TCT ACT ACA TAC ACC CCC GCA CAG Thr Gln Glu Val Ser Ile Pro Glu Ser Thr Thr Tyr Thr Pro Ala Gln | 245 | 250 | 255 | 768 |
| ACC TCC ACC CTT GTG TCC TCA AGC ACC AAG GAA GAC GCA GTG CAA ACG Thr Ser Thr Leu Val Ser Ser Ser Thr Lys Glu Asp Ala Val Gln Thr | 260 | 265 | 270 | 816 |
| CCG CCT AGG AAA CGA GCA CGA GGA GTC CAA CAG TCC CCT TGC AAC GCC Pro Pro Arg Lys Arg Ala Arg Gly Val Gln Gln Ser Pro Cys Asn Ala | 275 | 280 | 285 | 864 |
| TTG TGT GTG GCC CAC ATT GGA CCC GTG GAC AGT GGA AAC CAC AAC CTC Leu Cys Val Ala His Ile Gly Pro Val Asp Ser Gly Asn His Asn Leu | 290 | 295 | 300 | 912 |
| ATC ACT AAC AAT CAC GAC CAG CAC CAA AGA CGG AAC AAC AGT AAC AGT Ile Thr Asn Asn His Asp Gln His Gln Arg Arg Asn Asn Ser Asn Ser | 305 | 310 | 315 | 960 |
| TCA GCT ACG CCT ATA GTG CAA TTT CAA GGT GAA TCC AAT TGT TTA AAG Ser Ala Thr Pro Ile Val Gln Phe Gln Gly Glu Ser Asn Cys Leu Lys | 325 | 330 | 335 | 1008 |
| TGT TTT AGA TAT AGG CTA AAT GAC AGA CAC AGA CAT TTA TTT GAT TTA Cys Phe Arg Tyr Arg Leu Asn Asp Arg His Arg His Leu Phe Asp Leu | 340 | 345 | 350 | 1056 |
| ATA TCA TCA ACG TGG CAC TGG GCC TCC TCA AAG GCA CCA CAT AAA CAT Ile Ser Ser Thr Trp His Trp Ala Ser Ser Lys Ala Pro His Lys His | 355 | 360 | 365 | 1104 |
| GCC ATT GTA ACT GTA ACA TAT GAT AGT GAG GAA CAA AGG CAA CAG TTT Ala Ile Val Thr Val Thr Tyr Asp Ser Glu Glu Gln Arg Gln Gln Phe | 370 | 375 | 380 | 1152 |
| TTA GAT GTT GTA AAA ATA CCC CCT ACC ATT AGC CAC AAA CTG GGA TTT Leu Asp Val Val Lys Ile Pro Pro Thr Ile Ser His Lys Leu Gly Phe | 385 | 390 | 395 | 1200 |
| ATG TCA CTG CAC CTA TTG GTC GAC ATG GGA GCA CCA AAC ATT GGG AAG Met Ser Leu His Leu Leu Val Asp Met Gly Ala Pro Asn Ile Gly Lys | 405 | 410 | 415 | 1248 |
| TAT GTT ATG GCA GCA CAG TTA TAT GTT CTC CTG CAT CTG TAT CTA GCA Tyr Val Met Ala Ala Gln Leu Tyr Val Leu Leu His Leu Tyr Leu Ala | 420 | 425 | 430 | 1296 |
| CTA CAC AAG AAG TAT CCA TTC CTG AAT CTA CTA CAT ACA CCC CCG CAC Leu His Lys Lys Tyr Pro Phe Leu Asn Leu Leu His Thr Pro Pro His | 435 | 440 | 445 | 1344 |

| | |
|---|------|
| AGA CCT CCA CCC TTG TGT CCT CAA GCA CCA AGG AAG ACG CAG TGC AAA Arg Pro Pro Pro Leu Cys Pro Gln Ala Pro Arg Lys Thr Gln Cys Lys 450 455 460 | 1392 |
| CGC CGC CTA GGA AAC GAG CAC GAG GAG TCC AAC AGT CCC CTT GCA ACG Arg Arg Leu Gly Asn Glu His Glu Glu Ser Asn Ser Pro Leu Ala Thr 465 470 475 480 | 1440 |
| CCT TGT GTG TGG CCC ACA TTG GAC CCG TGG ACA GTG GAA ACC ACA ACC Pro Cys Val Trp Pro Thr Leu Asp Pro Trp Thr Val Glu Thr Thr Thr 485 490 495 | 1488 |
| TCA TCA CTA ACA ATC ACG ACC AGC ACC AAA GAC GGA ACA ACA GTA ACA Ser Ser Leu Thr Ile Thr Thr Ser Thr Lys Asp Gly Thr Thr Val Thr 500 505 510 | 1536 |
| GTT CAG CTA CGC CTA AGA TCT ATG GAA GTG GTG CCT GTA CAA ATA GCT Val Gln Leu Arg Leu Arg Ser Met Glu Val Val Pro Val Gln Ile Ala 515 520 525 | 1584 |
| GCA GGA ACA ACC AGC ACA TTC ATA CTG CCT GTT ATA ATT GCA TTT GTT Ala Gly Thr Thr Ser Thr Phe Ile Leu Pro Val Ile Ile Ala Phe Val 530 535 540 | 1632 |
| GTA TGT TTT GTT AGC ATC ATA CTT ATT GTA TGG ATA TCT GAG TTT ATT Val Cys Phe Val Ser Ile Ile Leu Ile Val Trp Ile Ser Glu Phe Ile 545 550 555 560 | 1680 |
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| TGG CTG GGT TTG TGG TTG TTA TGT GCC TTT ATT GTA GGG ATG TTG GGG Trp Leu Gly Leu Trp Leu Leu Cys Ala Phe Ile Val Gly Met Leu Gly 625 630 635 640 | 1920 |
| TTA TTA TTG ATG CAC TAT AGA GCT GTA CAA GGG GAT AAA CAC ACC AAA Leu Leu Leu Met His Tyr Arg Ala Val Gln Gly Asp Lys His Thr Lys 645 650 655 | 1968 |
| TGT AAG AAG TGT AAC AAA CAC AAC TGT AAT GAT GAT TAT GTA ACT ATG Cys Lys Lys Cys Asn Lys His Asn Cys Asn Asp Asp Tyr Val Thr Met 660 665 670 | 2016 |
| CAT TAT ACT ACT GAT GGT GAT TAT ATA TAT ATG AAT CCG CGG ATG GAA His Tyr Thr Thr Asp Gly Asp Tyr Ile Tyr Met Asn Pro Arg Met Glu 675 680 685 | 2064 |
| AGT GCA AAT GCC TCC ACG TCT GCA ACG ACC ATA GAC CAG TTG TGC AAG Ser Ala Asn Ala Ser Thr Ser Ala Thr Thr Ile Asp Gln Leu Cys Lys 690 695 700 | 2112 |
| ACG TTT AAT CTA TCT ATG CAT ACG TTG CAA ATT AAT TGT GTG TTT TGC Thr Phe Asn Leu Ser Met His Thr Leu Gln Ile Asn Cys Val Phe Cys 705 710 715 720 | 2160 |
| AAG AAT GCA CTG ACC ACA GCA GAG ATT TAT TCA TAT GCA TAT AAA CAC Lys Asn Ala Leu Thr Thr Ala Glu Ile Tyr Ser Tyr Ala Tyr Lys His 725 730 735 | 2208 |
| CTA AAG GTC CTG TTT CGA GGC GGC TAT CCA TAT GCA GCC TGC GCG TGC Leu Lys Val Leu Phe Arg Gly Gly Tyr Pro Tyr Ala Ala Cys Ala Cys | 2256 |

| 740 | 745 | 750 | |
|---|-----|-----|------|
| TGC CTA GAA TTT CAT GGA AAA ATA AAC CAA TAT AGA CAC TTT GAT TAT Cys Leu Glu Phe His Gly Lys Ile Asn Gln Tyr Arg His Phe Asp Tyr 755 760 765 | | | 2304 |
| GCT GGA TAT GCA ACA ACA GTT GAA GAA GAA ACT AAA CAA GAC ATC TTA Ala Gly Tyr Ala Thr Thr Val Glu Glu Glu Thr Lys Gln Asp Ile Leu 770 775 780 | | | 2352 |
| GAC GTG CTA ATT CGG TGC TAC CTG TGT CAC AAA CCG CTG TGT GAA GTA Asp Val Leu Ile Arg Cys Tyr Leu Cys His Lys Pro Leu Cys Glu Val 785 790 800 | | | 2400 |
| GAA AAG GTA AAA CAT ATA CTA ACC AAG GCG CGG TTC ATA AAG CTA AAT Glu Lys Val Lys His Ile Leu Thr Lys Ala Arg Phe Ile Lys Leu Asn 805 810 815 | | | 2448 |
| TGT ACG TGG AAG GGT CGC TGC CTA CAC TGC TGG ACA ACA TGC ATG GAA Cys Thr Trp Lys Gly Arg Cys Leu His Cys Trp Thr Thr Cys Met Glu 820 825 830 | | | 2496 |
| GAC ATG TTA CCC GCT AGC ATG CAT GGA AGA CAT GTT ACC CTA AAG GAT Asp Met Leu Pro Ala Ser Met His Gly Arg His Val Thr Leu Lys Asp 835 840 845 | | | 2544 |
| ATT GTA TTA GAC CTG CAA CCT CCA GAC CCT GTA GGG TTA CAT TGC TAT Ile Val Leu Asp Leu Gln Pro Pro Asp Pro Val Gly Leu His Cys Tyr 850 855 860 | | | 2592 |
| GAG CAA TTA GTA GAC AGC TCA GAA GAT GAG GTG GAC GAA GTG GAC GGA Glu Gln Leu Val Asp Ser Ser Glu Asp Glu Val Asp Glu Val Asp Gly 865 870 875 880 | | | 2640 |
| CAA GAT TCA CAA CCT TTA AAA CAA CAT TTC CAA ATA GTG ACC TGT TGC Gln Asp Ser Gln Pro Leu Lys Gln His Phe Gln Ile Val Thr Cys Cys 885 890 895 | | | 2688 |
| TGT GGA TGT GAC AGC AAC GTT CGA CTG GTT GTG CAG TGT ACA GAA ACA Cys Gly Cys Asp Ser Asn Val Arg Leu Val Val Gln Cys Thr Glu Thr 900 905 910 | | | 2736 |
| GAC ATC AGA GAA GTG CAA CAG CTT CTG TTG GGA ACA CTA AAC ATA GTG Asp Ile Arg Glu Val Gln Gln Leu Leu Leu Gly Thr Leu Asn Ile Val 915 920 925 | | | 2784 |
| TGT CCC ATC TGC GCA CCG AAG ACC CGA TCG ATG GCG GAC GAT TCA GGT Cys Pro Ile Cys Ala Pro Lys Thr Arg Ser Met Ala Asp Asp Ser Gly 930 935 940 | | | 2832 |
| ACA GAA AAT GAG GGG TCT GGG TGT ACA GGA TGG TTT ATG GTA GAA GCT Thr Glu Asn Glu Gly Ser Gly Cys Thr Gly Trp Phe Met Val Glu Ala 945 950 955 960 | | | 2880 |
| ATA GTG CAA CAC CCA ACA GGT ACA CAA ATA TCA GAC GAT GAG GAT GAG Ile Val Gln His Pro Thr Gly Thr Gln Ile Ser Asp Asp Glu Asp Glu 965 970 975 | | | 2928 |
| GAG GTG GAG GAC AGT GGG TAT GAC ATG GTG GAC TTT ATT GAT GAC AGC Glu Val Glu Asp Ser Gly Tyr Asp Met Val Asp Phe Ile Asp Asp Ser 980 985 990 | | | 2976 |
| AAT ATT ACA CAC AAT TCA CTG GAA GCA CAG GCA TTG TTT AAC AGG CAG Asn Ile Thr His Asn Ser Leu Glu Ala Gln Ala Leu Phe Asn Arg Gln 995 1000 1005 | | | 3024 |
| GAG GCG GAC ACC CAT TAT GCG ACT GTG CAG GAC CTA AAA CGA AAG TAT Glu Ala Asp Thr His Tyr Ala Thr Val Gln Asp Leu Lys Arg Lys Tyr 1010 1015 1020 | | | 3072 |
| TTA GGT AGT CCA TAT GTT AGT CCT ATA AAC ACT ATA GCC GAG GCA GTG Leu Gly Ser Pro Tyr Val Ser Pro Ile Asn Thr Ile Ala Glu Ala Val 1025 1030 1035 1040 | | | 3120 |

| | |
|---|------|
| GAA AGT GAA ATA AGT CCA CGA TTG GAC GCC ATT AAA CTT ACA AGA CAG Glu Ser Glu Ile Ser Pro Arg Leu Asp Ala Ile Lys Leu Thr Arg Gln 1045 1050 1055 | 3168 |
| CCA AAA AAG GTA AAG CGA CGG CTG TTT CAA ACC AGG GAA CTA ACG GAC Pro Lys Lys Val Lys Arg Arg Leu Phe Gln Thr Arg Glu Leu Thr Asp 1060 1065 1070 | 3216 |
| AGT GGA TAT GGC TAT TCT GAA GTG GAA GCT GGA ACG GGA ACG CAG GTA Ser Gly Tyr Gly Tyr Ser Glu Val Glu Ala Gly Thr Gly Thr Gln Val 1075 1080 1085 | 3264 |
| GAG AAA CAT GGC GTA CCG GAA AAT GGG GGA GAT GGT CAG GAA AAG GAC Glu Lys His Gly Val Pro Glu Asn Gly Gly Asp Gly Gln Glu Lys Asp 1090 1095 1100 | 3312 |
| ACA GGA AGG GAC ATA GAG GGG GAG GAA CAT ACA GAG GCG GAA GCG CCC Thr Gly Arg Asp Ile Glu Gly Glu Glu His Thr Glu Ala Glu Ala Pro 1105 1110 1115 1120 | 3360 |
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| TTA AAA TGT AAA GAT TTA CGG GCA GCA TTA CTT GGT AAG TTT AAA GAA Leu Lys Cys Lys Asp Leu Arg Ala Leu Leu Gly Lys Phe Lys Glu 1140 1145 1150 | 3456 |
| TGC TTT GGG CTG TCT TTT ATA GAT TTA ATT AGG CCA TTT AAA AGT GAT Cys Phe Gly Leu Ser Phe Ile Asp Leu Ile Arg Pro Phe Lys Ser Asp 1155 1160 1165 | 3504 |
| AAA ACA ACA TGT TTA GAT TGG GTG GTA GCA GGG TTT GGT ATA CAT CAT Lys Thr Thr Cys Leu Asp Trp Val Val Ala Gly Phe Gly Ile His His 1170 1175 1180 | 3552 |
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| TTA TTA AGA TTT AAA GTA AAT AAA AGT AGA AGT ACC GTT GCA CGT ACA Leu Leu Arg Phe Lys Val Asn Lys Ser Arg Ser Thr Val Ala Arg Thr 1220 1225 1230 | 3696 |
| CTT GCA ACG CTA TTA AAT ATA CCT GAA AAC CAA ATG TTA ATA GAG CCA Leu Ala Thr Leu Leu Asn Ile Pro Glu Asn Gln Met Leu Ile Glu Pro 1235 1240 1245 | 3744 |
| CCA AAA ATA CAA AGT GGT GTT GCA GCC CTG TAT TGG TTT CGT ACA GGT Pro Lys Ile Gln Ser Gly Val Ala Ala Leu Tyr Trp Phe Arg Thr Gly 1250 1255 1260 | 3792 |
| ATA TCA AAT GCC AGT ACA GTT ATA GGG GAA GCA CCA GAA TGG ATA ACA Ile Ser Asn Ala Ser Thr Val Ile Gly Glu Ala Pro Glu Trp Ile Thr 1265 1270 1275 1280 | 3840 |
| CGC CAA ACA GTT ATT GAA CAC GGG TTG GCA GAC AGT CAG TTT AAA TTA Arg Gln Thr Val Ile Glu His Gly Leu Ala Asp Ser Gln Phe Lys Leu 1285 1290 1295 | 3888 |
| ACA GAA ATG GTG CAG TGG GCG TAT GAT AAT GAC ATA TGC GAG GAG AGT Thr Glu Met Val Gln Trp Ala Tyr Asp Asn Asp Ile Cys Glu Glu Ser 1300 1305 1310 | 3936 |
| GAA ATT GCA TTT GAA TAT GCA CAA AGG GGA GAT TTT GAT TCT AAT GCA Glu Ile Ala Phe Glu Tyr Ala Gln Arg Gly Asp Phe Asp Ser Asn Ala 1315 1320 1325 | 3984 |
| CGA GCA TTT TTA AAT AGC AAT ATG CAG GCA AAA TAT GTG AAA GAT TGT Arg Ala Phe Leu Asn Ser Asn Met Gln Ala Lys Tyr Val Lys Asp Cys 1330 1335 1340 | 4032 |

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| 1330 | 1335 | 1340 | |
|---|-----------------------------|------|--|
| GCA ACT ATG TGT AGA CAT TAT AAA CAT | GCA GAA ATG AGG AAG ATG TCT | 4080 | |
| Ala Thr Met Cys Arg His Tyr Lys His | Ala Glu Met Arg Lys Met Ser | | |
| 1345 | 1355 1360 | | |
| ATA AAA CAA TGG ATA AAA CAT AGG GGT TCT AAA ATA GAA GGC ACA GGA | 4128 | | |
| Ile Lys Gln Trp Ile Lys His Arg Gly Ser Lys Ile Glu Gly Thr Gly | | | |
| 1365 | 1370 1375 | | |
| AAT TGG AAA CCA ATT GTA CAA TTC CTA CGA CAT CAA AAT ATA GAA TTC | 4176 | | |
| Asn Trp Lys Pro Ile Val Gln Phe Leu Arg His Gln Asn Ile Glu Phe | | | |
| 1380 | 1385 1390 | | |
| ATT CCT TTT TTA ACT AAA TTT AAA TTA TGG CTG CAC GGT ACG CCA AAA | 4224 | | |
| Ile Pro Phe Leu Thr Lys Phe Lys Leu Trp Leu His Gly Thr Pro Lys | | | |
| 1395 | 1400 1405 | | |
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| 1410 | 1415 1420 | | |
| TTT TGT ATG AGT TTA ATA AGC TTT CTA GGA GGT ACA GTT ATT AGT CAT | 4320 | | |
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| Val Ala Leu Leu Asp Asp Ala Thr Gln Pro Cys Trp Ile Tyr Met Asp | | | |
| 1460 | 1465 1470 | | |
| ACA TAT ATG AGA AAT TTG TTA GAT GGT AAT CCT ATG AGT ATT GAC AGA | 4464 | | |
| Thr Tyr Met Arg Asn Leu Leu Asp Gly Asn Pro Met Ser Ile Asp Arg | | | |
| 1475 | 1480 1485 | | |
| AAG CAT AAA GCA TTG ACA TTA ATT AAA TGT CCA CCT CTG CTA GTA ACG | 4512 | | |
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| 1490 | 1495 1500 | | |
| TCC AAC ATA GAT ATT ACT AAA GAA GAT AAA TAT AAG TAT TTA CAT ACT | 4560 | | |
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| 1505 | 1510 1515 1520 | | |
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| Gly Asn Ala Val Tyr Glu Leu Ser Asn Thr Asn Trp Lys Cys Phe Phe | | | |
| 1540 | 1545 1550 | | |
| GAA AGA CTG TCG TCA AGC CTA GAC ATT CAG GAT TCT GAG GAC GAG GAA | 4704 | | |
| Glu Arg Leu Ser Ser Ser Leu Asp Ile Gln Asp Ser Glu Asp Glu Glu | | | |
| 1555 | 1560 1565 | | |
| GAT GGA AGC AAT AGC CAA GCG TTT AGA TGC GTG CCA GGA ACA GTT GTT | 4752 | | |
| Asp Gly Ser Asn Ser Gln Ala Phe Arg Cys Val Pro Gly Thr Val Val | | | |
| 1570 | 1575 1580 | | |
| AGA ACT TTA TGAGGTACC | 4770 | | |
| Arg Thr Leu | | | |
| 1585 | | | |

CLAIMS:

1. A polyprotein construct comprising at least two amino acid sequences fused directly or indirectly together, each of said sequences being the sequence of an early ORF protein of papillomavirus (PV) or an immunogenic variant or fragment thereof, and at least one of said sequences being other than the E6 or E7 protein sequence or an immunogenic variant or fragment thereof.
2. A polyprotein construct according to claim 1, wherein said sequences are sequences of early ORF proteins of human PV, or immunogenic variants or fragments thereof.
3. A polyprotein construct according to claim 2, wherein said early ORF proteins are selected from the group consisting of the E1, E2, E3, E4, E5 (E5a, E5b), E6, E7 and E8 proteins of PV.
4. A polyprotein construct according to any of claims 1 to 3, selected from the group consisting of:
 - (a) E6/E4
 - (b) E6/E5a/E4
 - (c) E6/E7/E4
 - (d) E6/E7/E5a/E4
 - (e) E6/E7/E1/E4
 - (f) E6/E7/E5a/E1/E4
 - (g) E6/E7/E5a/E1/E2/E4
 - (h) E6/E7/E5a/E5b/E1/E2/E4
 - (i) E2/E5b
 - (j) E2/E1/E5b
 - (k) E2/E5a/E5b
 - (l) E2/E1/E5a/E5b

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- (m) E2/E4/E5a/E5b/E6/E7/E1
- (n) E2/E3/E4/E5/E8/E6/E7/E1.

5. A polyprotein construct according to claim 1, further comprising one or more linker sequences between and/or before and/or after said amino acid sequences.
6. A polyprotein construct according to claim 5, wherein said linker sequence(s) comprise from 1 to 5 amino acid residues.
7. A polyprotein construct according to claim 1, further comprising a tag protein or peptide moiety fused or otherwise coupled thereto.
8. A polyprotein construct according to claim 7, wherein said tag moiety is selected from the group consisting of (his)₆, glutathione-S-transferase (GST) and FLAG.
9. A polyprotein construct according to claim 1, further comprising an adjuvant moiety fused or otherwise coupled thereto.
10. A polyprotein construct according to claim 9, wherein said adjuvant moiety is selected from diphtheria toxin, cholera toxin and *E. coli* heat labile toxin (LT) and non-toxic derivatives thereof such as the holotoxoid or B sub-unit of cholera toxin or LT.
11. A polyprotein construct according to claim 1, further comprising a lipid binding region.
12. A polyprotein construct according to claim 11, wherein said lipid binding region is an influenza haemagglutinin tail.

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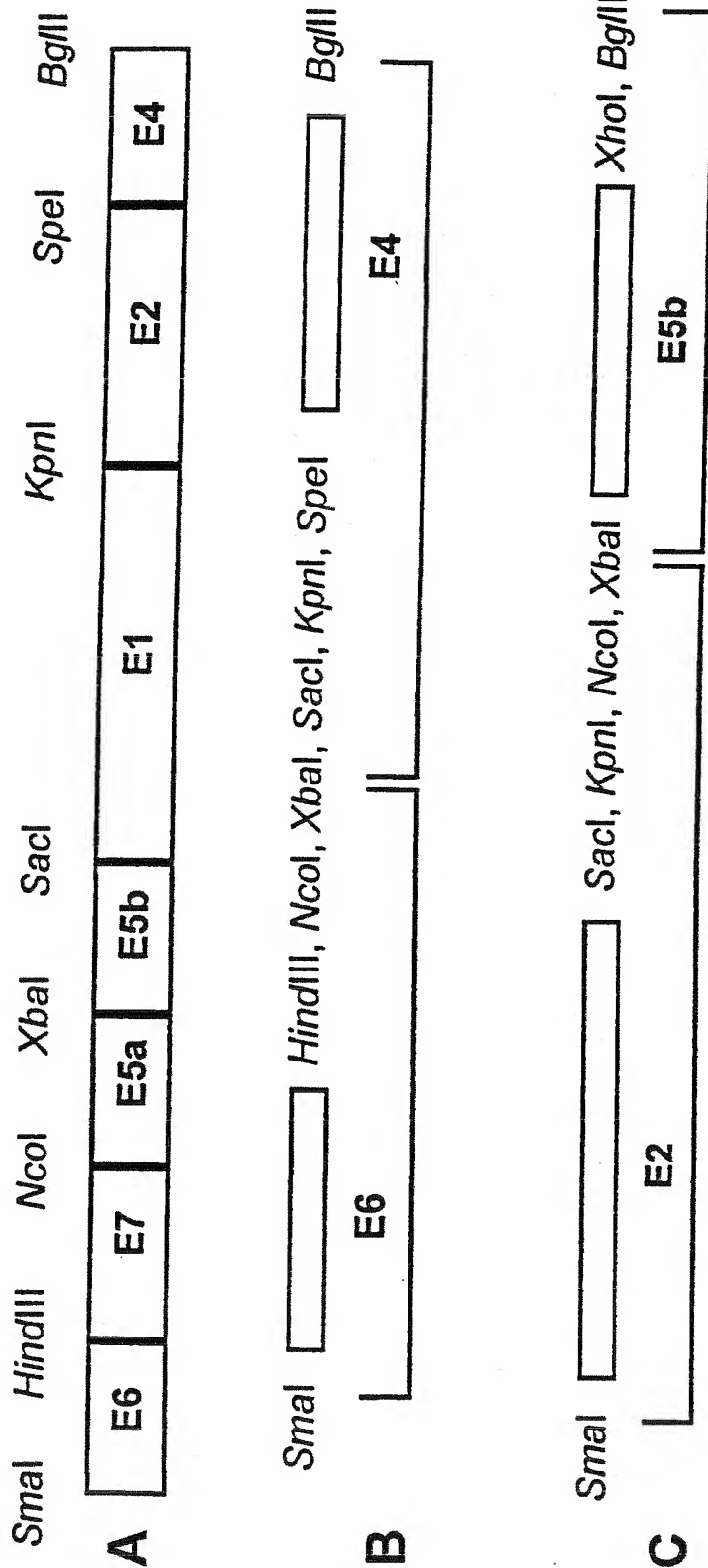
13. A composition for eliciting a humoral and/or cellular immune response against papillomavirus in a host animal, said composition comprising an immunologically effective amount of a polyprotein construct according to any of claims 1 to 12, together with a pharmaceutically acceptable carrier and/or diluent.
14. A vaccine composition according to claim 13, further comprising an adjuvant.
15. A method for eliciting a humoral and/or cellular response against papillomavirus in a host animal, which method comprises administering to the host animal an immunologically effective amount of a polyprotein construct according to any of claims 1 to 12.
16. A method according to claim 15, wherein said polyprotein construct is administered in a composition together with a pharmaceutically acceptable carrier and/or diluent.
17. A method according to claim 16, wherein said composition further comprises an adjuvant.
18. A method according to any of claims 15 to 17, wherein said host animal is a human.
19. Use of a polyprotein construct according to any of claims 1 to 12, in eliciting an immune response against papillomavirus in a host animal.
20. A nucleic acid molecule which encodes a polyprotein construct according to any of claims 1 to 12.
21. A recombinant DNA molecule comprising an expression control sequence operatively linked to a nucleic acid molecule according to claim 20.

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22. A recombinant DNA molecule according to claim 21, wherein said expression control sequence comprises promoter and initiator sequences, the sequence of nucleotides encoding the polyprotein construct being located in a single translational frame 3' to the promoter and initiator sequences, and a termination sequence located 3' to said sequence of nucleotides.
23. A recombinant DNA cloning vehicle or vector comprising a recombinant DNA molecule according to claim 21 or claim 22.
24. A recombinant DNA cloning vehicle or vector according to claim 23, wherein said vector is a plasmid.
25. A host cell transfected or transformed with a recombinant DNA molecule according to claim 21 or claim 22, or a recombinant DNA cloning vehicle or vector according to claim 23 or claim 24.
26. A host cell according to claim 25, wherein said host cell is *E. coli*.
27. A recombinant polyprotein construct prepared by expression in a host cell according to claim 25 or claim 26.
28. A composition comprising a nucleic acid molecule according to claim 20, together with a pharmaceutically acceptable carrier and/or diluent.
29. A method for eliciting an immune response against PV in a host animal, which method comprises administering to the host animal an immunologically effective amount of a nucleic acid molecule according to claim 20.
30. Use of a nucleic acid molecule according to claim 20 in eliciting an immune response against PV in a host animal.

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Figure 1



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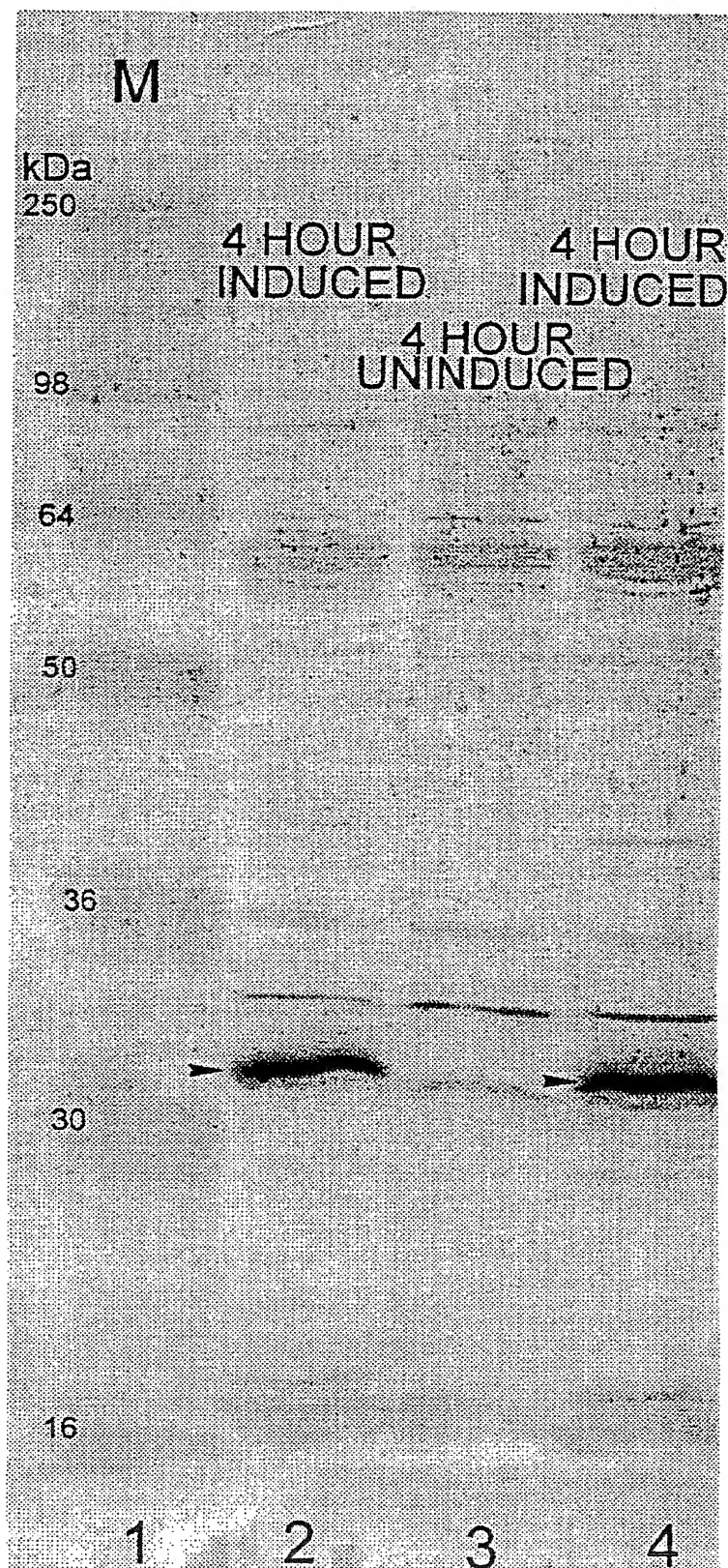
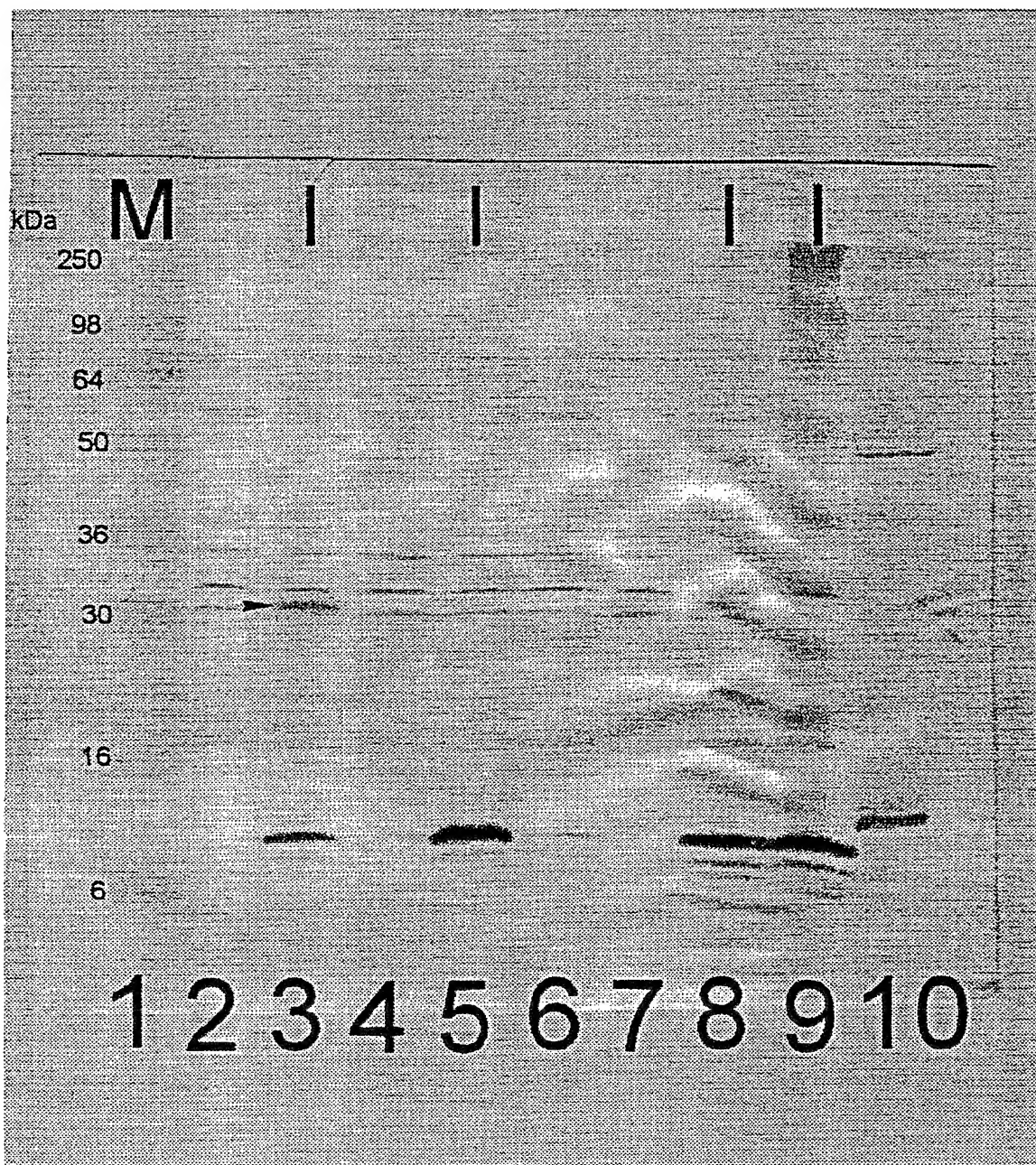


FIGURE 2

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Figure 3



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ANTI-HEXAHIS (DIANOVA) Mab

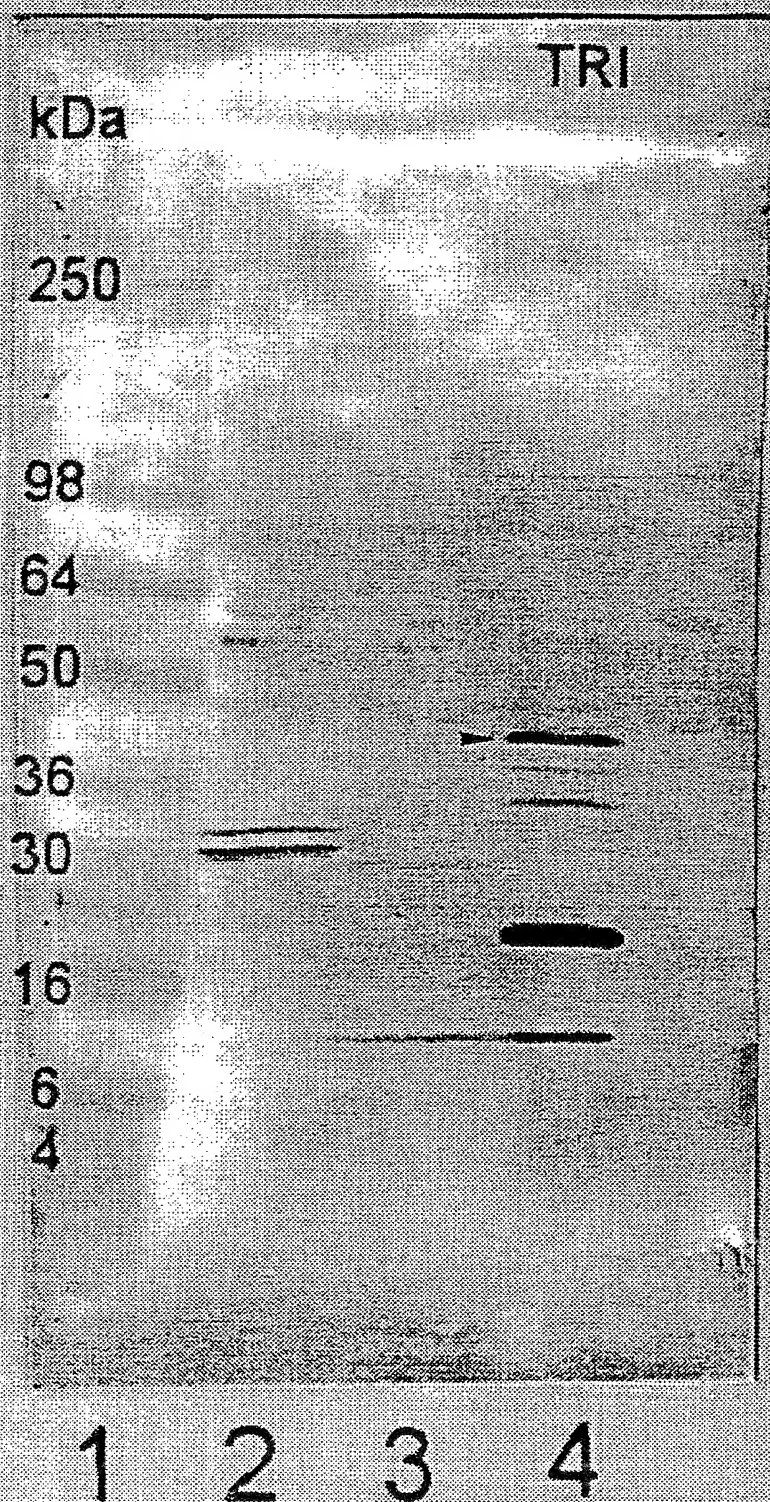


Figure 4.

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ANTI-E4 PEPTIDE

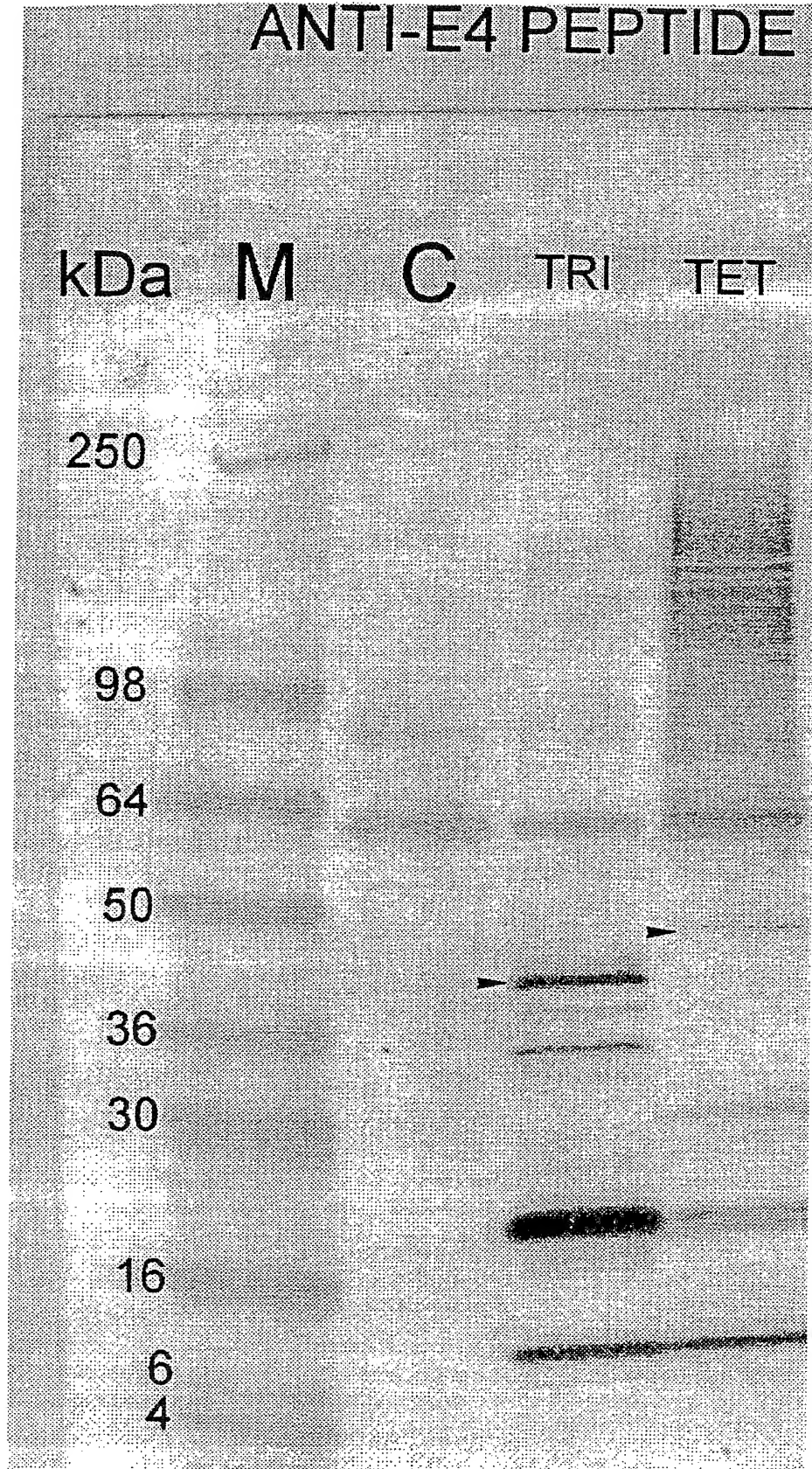
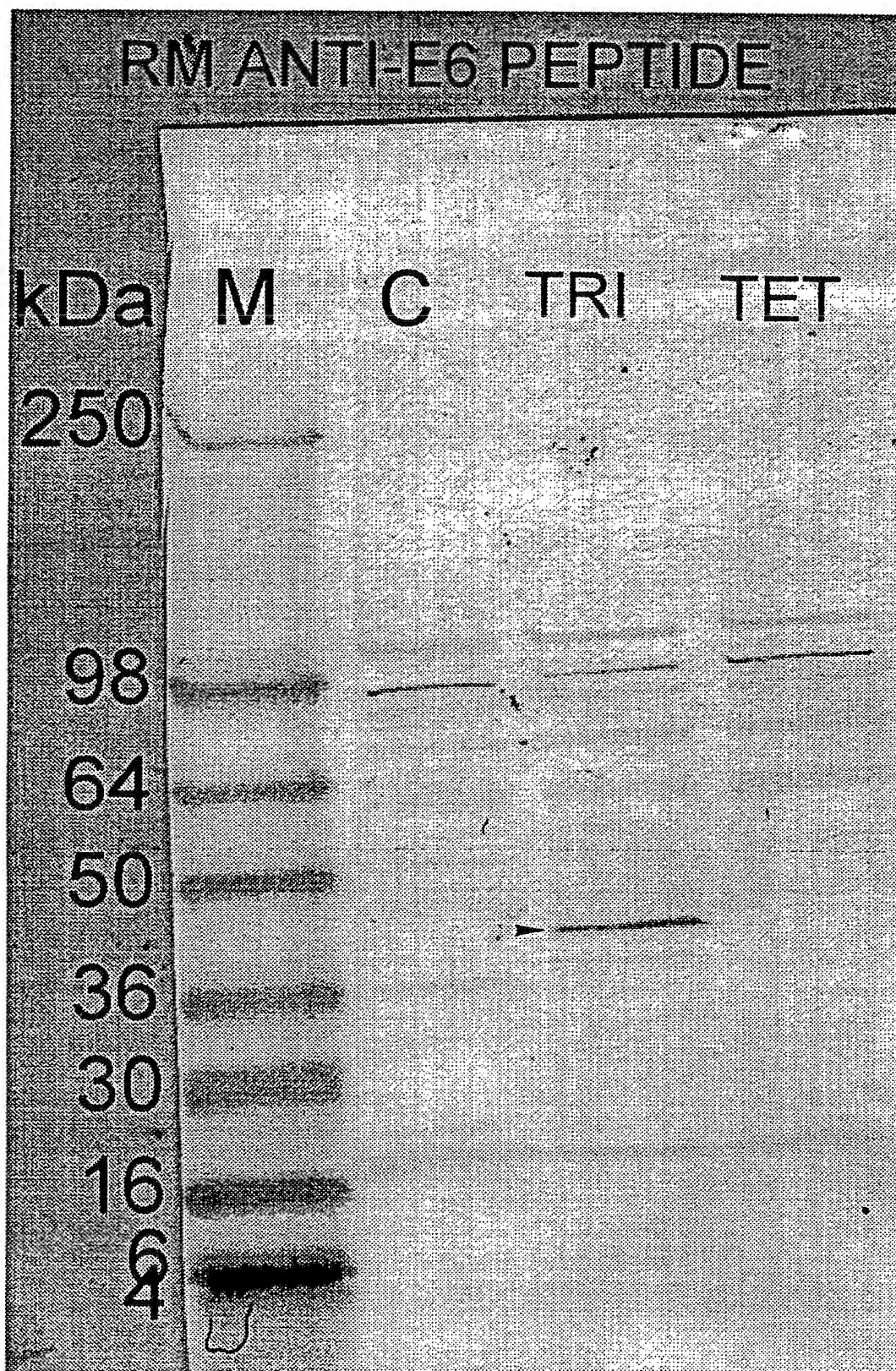


Figure 5.

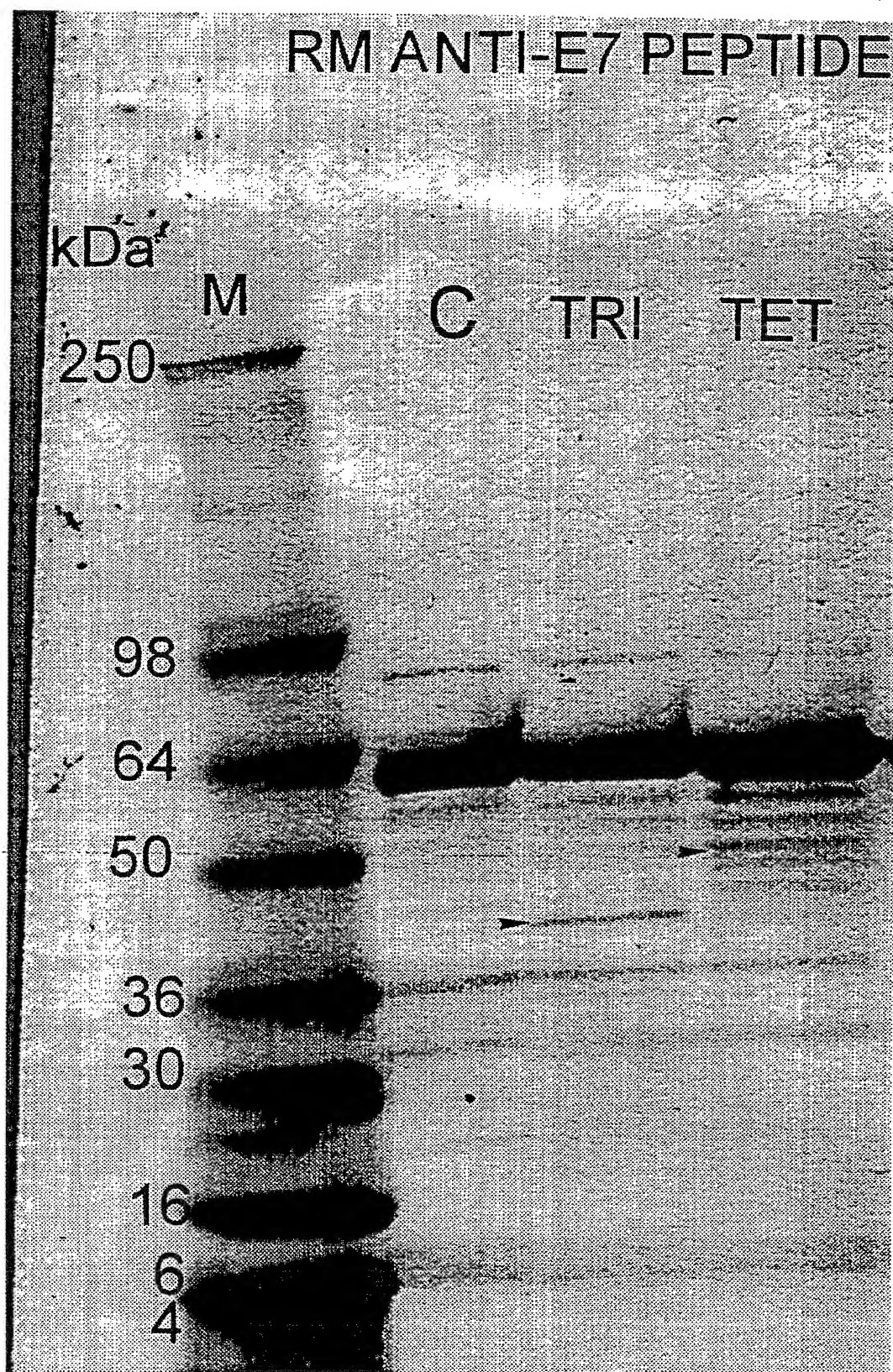
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Figure 6



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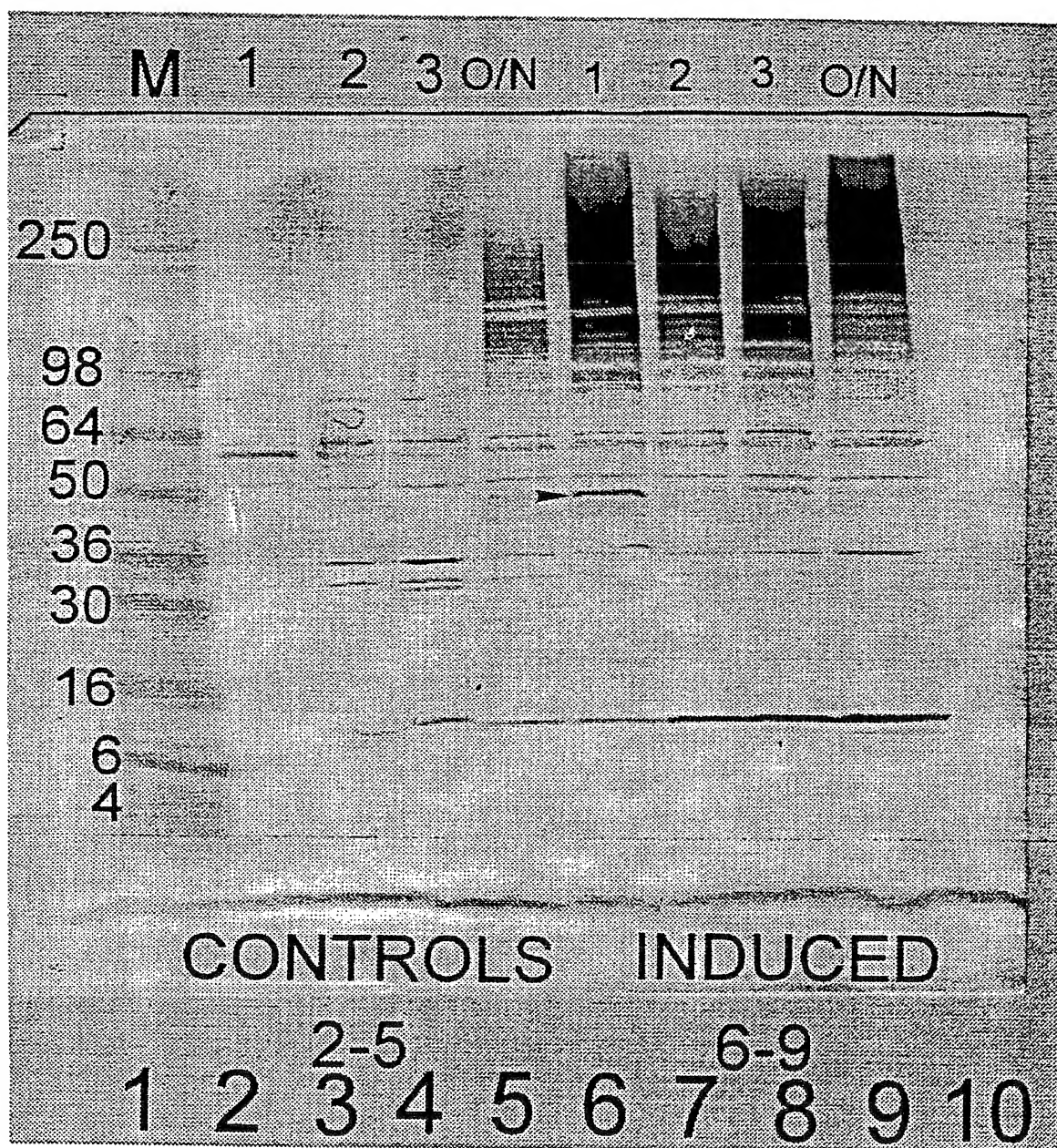
Figure 7



SUBSTITUTE SHEET (RULE 26)

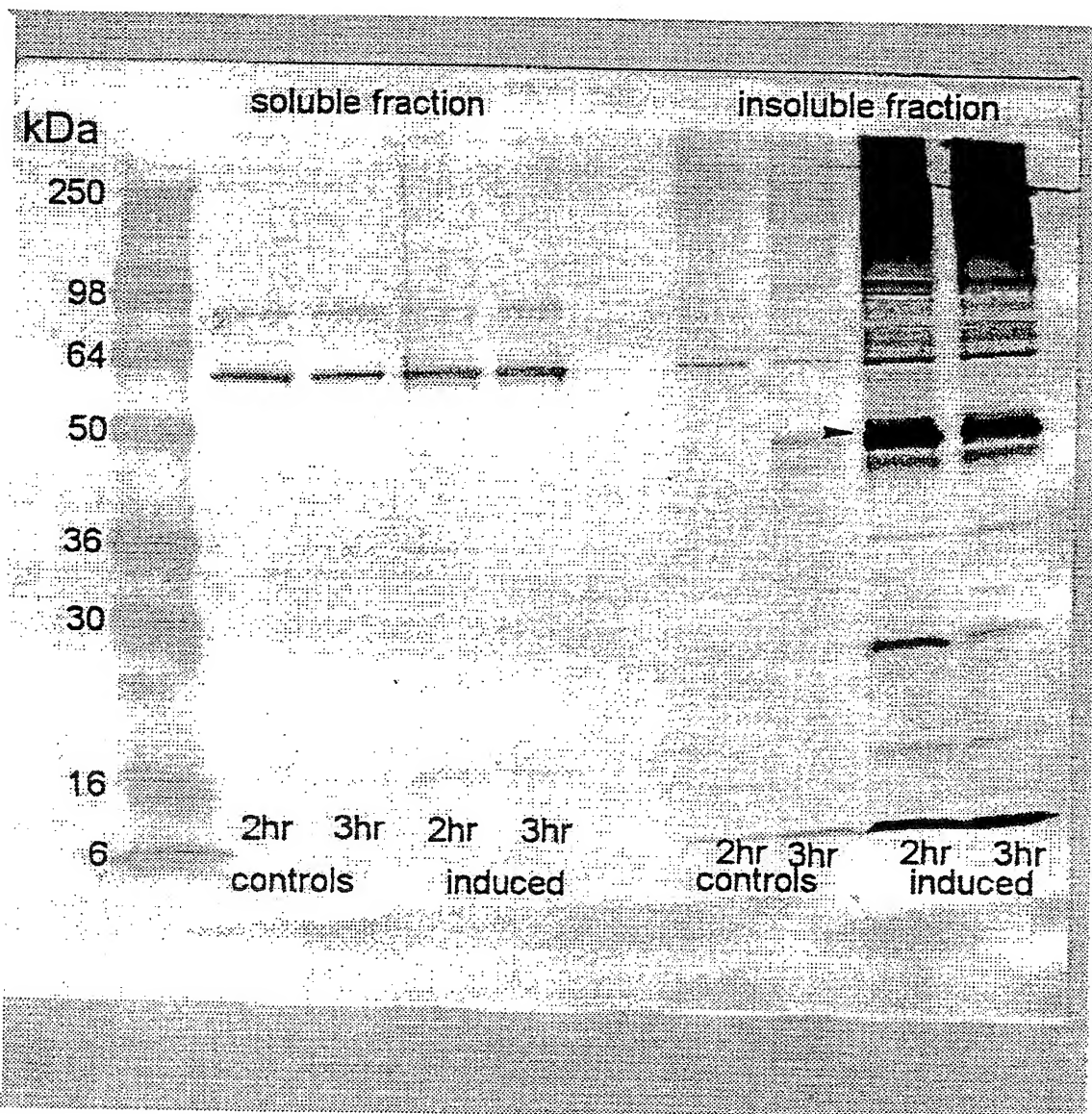
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Figure 8



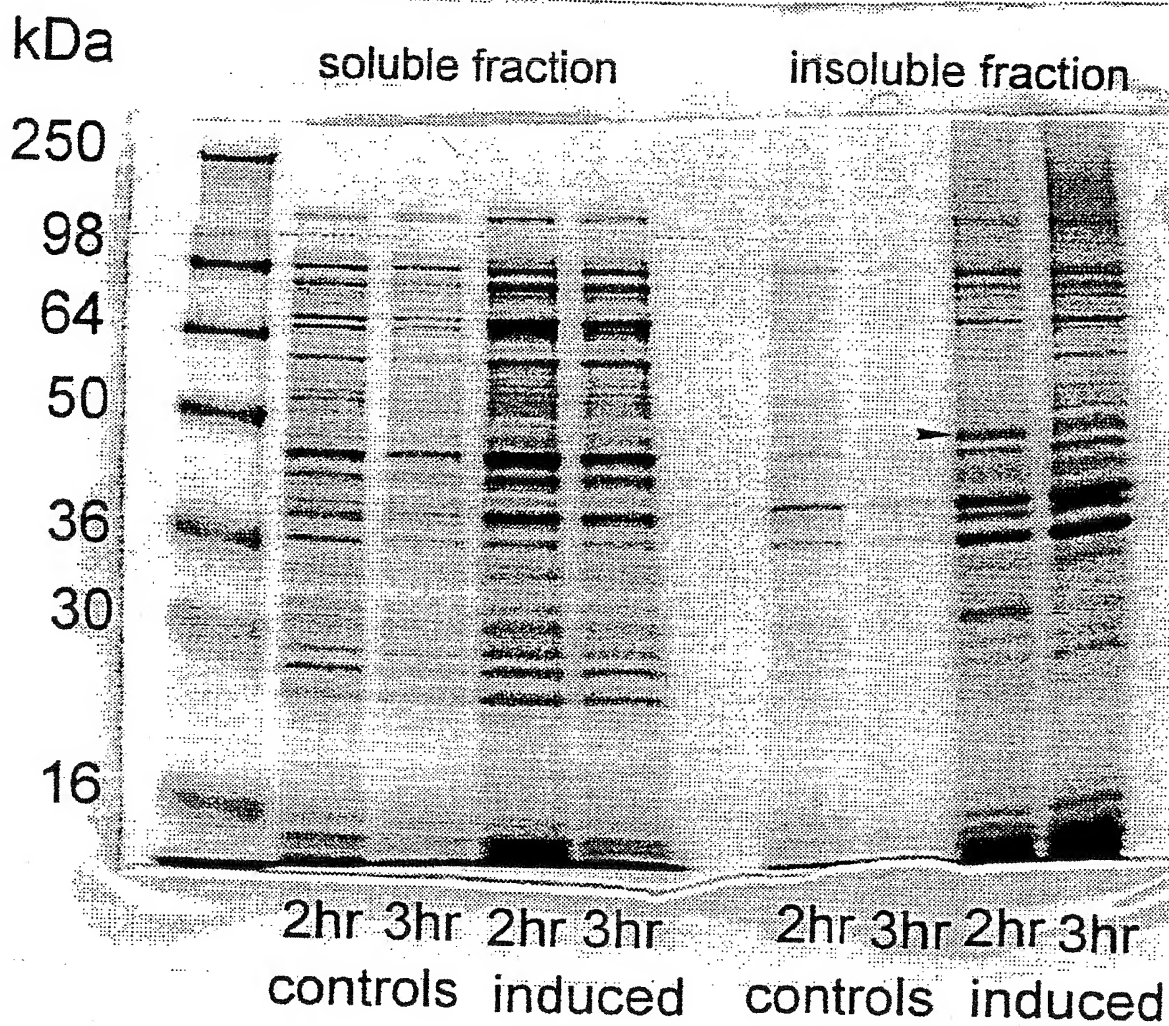
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Figure 9



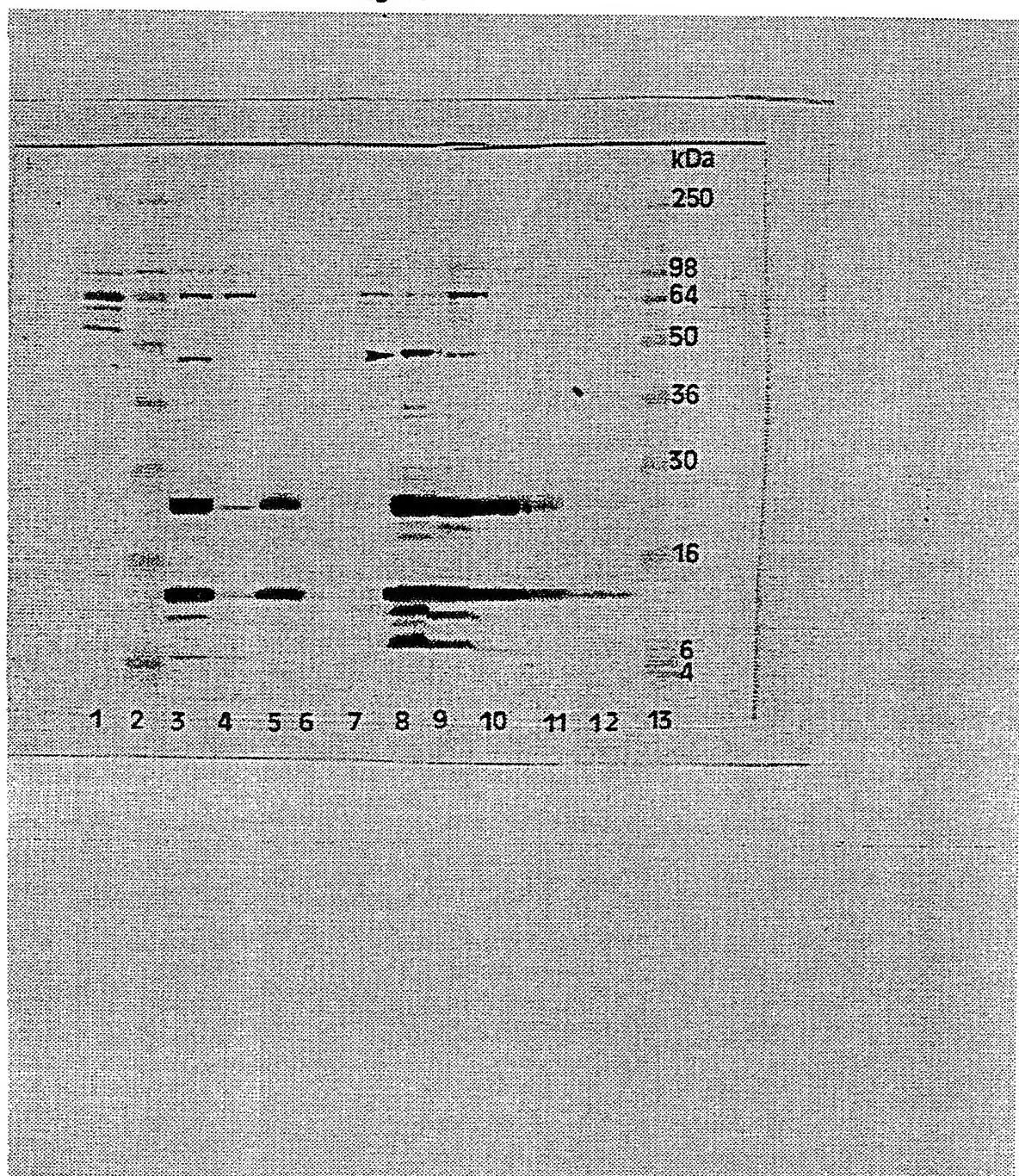
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Figure 10



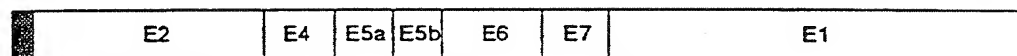
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Figure 11



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Figure 12



100 aa



hexaHis Tag encoded by pTrcHisA

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 96/00473

| | | | | | | | | | | | | | | | | | | | | | | |
|--|--|---|--|--|-----|---|-----|--|-----|--|-----|---|-----|--|-----|--|-----|---|-----|--|--|--|
| A. CLASSIFICATION OF SUBJECT MATTER | | | | | | | | | | | | | | | | | | | | | | |
| Int Cl ⁶ : C07K 14/025; C12N 15/37, 15/86, 5/10; A61K 39/12, 31/73 | | | | | | | | | | | | | | | | | | | | | | |
| According to International Patent Classification (IPC) or to both national classification and IPC | | | | | | | | | | | | | | | | | | | | | | |
| B. FIELDS SEARCHED | | | | | | | | | | | | | | | | | | | | | | |
| Minimum documentation searched (classification system followed by classification symbols) IPC ⁶ : C07K, C12N, A61K. Chemical Abstracts. All through Electronic Databases | | | | | | | | | | | | | | | | | | | | | | |
| Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched | | | | | | | | | | | | | | | | | | | | | | |
| Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) DERWENT Databases: WPAT & JAPIO. Search terms: See extra sheet. | | | | | | | | | | | | | | | | | | | | | | |
| C. DOCUMENTS CONSIDERED TO BE RELEVANT | | | | | | | | | | | | | | | | | | | | | | |
| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. | | | | | | | | | | | | | | | | | | | | |
| P, Y | DE 4435907 (GUTZMANN et al), 11 April 1996, IPC ⁶ C07K 14/37, 14/01, 14/08; A61K 38/16 See claims, especially claims 9 and 10 | 1-3 | | | | | | | | | | | | | | | | | | | | |
| X | TANIGUCHI & YASUMOTO: "A Major Transcript of Human Papillomavirus Type 16 in Transformed NIH 3T3 Cells contains Polycistronic mRNA encoding E7, E5, and E1 [^] E4 Fusion Gene". Virus Genes, 3(3), pp 221-233, 1990. See abstract, figures 3 and 6, p 229 lines 4-10 and 15-16 | 1-3 | | | | | | | | | | | | | | | | | | | | |
| <input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C <input checked="" type="checkbox"/> See patent family annex | | | | | | | | | | | | | | | | | | | | | | |
| <p>* Special categories of cited documents:</p> <table border="0"> <tr> <td>"A"</td> <td>document defining the general state of the art which is not considered to be of particular relevance</td> <td>"T"</td> <td>later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td> </tr> <tr> <td>"E"</td> <td>earlier document but published on or after the international filing date</td> <td>"X"</td> <td>document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td> </tr> <tr> <td>"L"</td> <td>document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td> <td>"Y"</td> <td>document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td> </tr> <tr> <td>"O"</td> <td>document referring to an oral disclosure, use, exhibition or other means</td> <td>"&"</td> <td>document member of the same patent family</td> </tr> <tr> <td>"P"</td> <td>document published prior to the international filing date but later than the priority date claimed</td> <td></td> <td></td> </tr> </table> | | | "A" | document defining the general state of the art which is not considered to be of particular relevance | "T" | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention | "E" | earlier document but published on or after the international filing date | "X" | document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone | "L" | document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "Y" | document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art | "O" | document referring to an oral disclosure, use, exhibition or other means | "&" | document member of the same patent family | "P" | document published prior to the international filing date but later than the priority date claimed | | |
| "A" | document defining the general state of the art which is not considered to be of particular relevance | "T" | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention | | | | | | | | | | | | | | | | | | | |
| "E" | earlier document but published on or after the international filing date | "X" | document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone | | | | | | | | | | | | | | | | | | | |
| "L" | document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "Y" | document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art | | | | | | | | | | | | | | | | | | | |
| "O" | document referring to an oral disclosure, use, exhibition or other means | "&" | document member of the same patent family | | | | | | | | | | | | | | | | | | | |
| "P" | document published prior to the international filing date but later than the priority date claimed | | | | | | | | | | | | | | | | | | | | | |
| Date of the actual completion of the international search 10 September 1996 | | Date of mailing of the international search report 18.09.96 | | | | | | | | | | | | | | | | | | | | |
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INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 96/00473

| C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT | | |
|--|---|-----------------------|
| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| X | ROHLFS et al: "Viral Transcription in Human Keratinocyte Cell Lines Immortalized by Human Papillomavirus Type-16". Virology, 183, pp 331-342 (1991). See Figure 1; page 334, column 2, lines 5-9, 13-15 and 19-20; page 335 column 1, lines 26-27 and column 2 lines 2-10 | 1-4 |
| X | CHIANG et al: "An E1M ⁺ E2C Fusion Protein Encoded by Human Papillomavirus Type 11 Is a Sequence-Specific Transcription Repressor". Journal of Virology, 65(6), pp 3317-3329, 1991. See abstract, p 3318, column 2, 2nd full paragraph, Figures 1 and 2, p 3321, column 1, 1st full sentence, column 2, line 3 - p 3322, column 1, line 2, column 2 lines 2-5, p 3323, column 1, 1st full paragraph, p 3326, column 2 lines 5-9 and 1st 2 sentences of 1st full paragraph | 1-4, 20-22 |
| X | LAMBERTI et al: "Transcriptional activation by the papillomavirus E6 zinc finger oncoprotein". The EMBO Journal, 9 (6), pp 1907-1913, 1990. See abstract, figure 1, p 1912, 2nd paragraph of "Constructions" | 1, 5, 20-22 |
| X | WO 92/11290 (CETUS CORPORATION), 9 July 1992, IPC ⁵ C07K 13/00, 15/18; A61K 37/10; G01N 33/569, 33/68; C12Q 1/18, 1/70. See abstract, p 6 lines 3-11, page 7 line 12 - page 12, line 9, claims | 1-3, 13-20 |
| X | TOMITA & SIMIZU: "Translational properties of the human papillomavirus type-6 L1-coding mRNA". Gene, 133, pp 223-225, 1993. See in particular figure 1B #3 | 1-3, 5, 20, 21 |
| X | WO 94/12629 (BAYLOR COLLEGE OF MEDICINE) 9 June 1994, IPC ⁵ C12N 15/00; A61K 31/70. See abstract, p 3 lines 16-31, p 7 line 19 - p8 line 7, p11 lines 1-15, p 16 lines 35-36, Example 1 (on p 27), claim 1, Figure 1 | 1-3, 5, 20, 21 |

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Box

Search terms used:

WPAT and JAPIO search

SS1 : PAPILLOMAVIRUS## OR PAPILLOMA(W)VIRUS##

SS2 : EARLY (3N)(ORF OR OPEN(W)READING(W)FRAME# OR PROTEIN# OR POLYPEPTIDE#)

SS3 : 1 AND 2

SS4 : E1# OR E2# OR E3# OR E4# OR E5# OR E6# OR E7# OR E8#

SS5 : 1 AND 4

SS 6 : 3 OR 5

Search terms used:

Chemical Abstracts Search

L1 : S EARLY (3N) (ORF OR OPEN()READING()FRAME# OR PROTEIN# OR POLYPEPTIDE#)/IT

L2 : S PAPILLOMAVIRUS?/IT OR PAPILLOMA()VIRUS##/IT

L3 : S (E1# OR E2# OR E3# OR E4# OR E5# OR E6# OR E7# OR E8#)/IT

L4 : S L1 AND L2

L5 : S L3 (L) L2

L6 : S (FUS## OR FUSI##)/IT

L7 : S L6 (L) L5

L8 : S L6(L) L4

L9 : S L7 OR L8

Information on patent family members

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